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GENOMICS

AAAS

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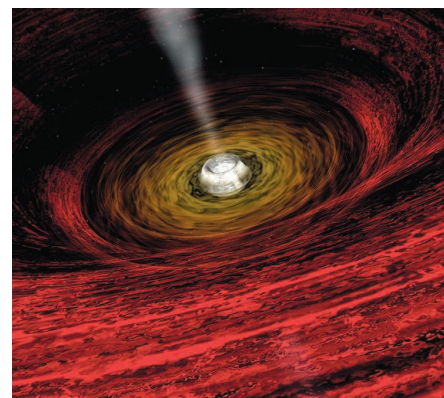
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Human colon cancer (at lower center) identified by a colored barium x-ray shown overlaid with a representation of a genetic sequence. Genome sequence analysis of human tumors has uncovered an array of genetic alterations that help drive tumor growth—information that may lead to more effective cancer therapies. See the special section beginning on page 1539.

Image: Mehau Kulyk/Science Source

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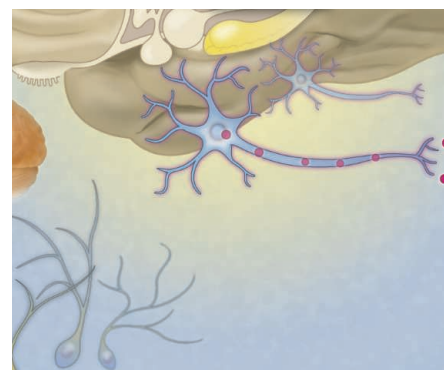
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A metabolite specific to certain cancers, and of therapeutic interest, exists in two forms, only one of which is oncogenic.

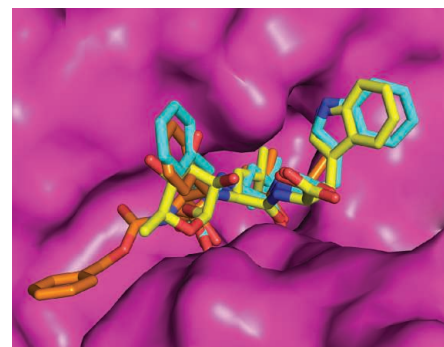
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The majority of food crops require pollination to set fruit with the honeybee providing a pollination workhorse, with both feral and managed populations an integral component of crop management (see the Perspective by **Tylianakis**, published online 28 February). **Garibaldi *et al.*** (p. 1608, published online 28 February) now show that wild pollinators are also a vital part of our crop systems. In more than 40 important crops grown worldwide, wild pollinators improved pollination efficiency, increasing fruit set by twice that facilitated by honeybees. **Burkle *et al.*** (p. 1611, published online 28 February) took advantage of one of the most thorough and oldest data sets available on plant-pollinator interaction networks and recollected data on plant-pollinator interactions after more than 120 years of climate change and landscape alteration. The historical data set consists of observations collected by Charles Robertson near Carlinville, Illinois (USA), in the late 1800s on the phenology of plants and their pollinating insects, as well as information about which plants and pollinators interacted with one another. Many sites were revisited in the early 1970s and in 2009 and 2010 to collect similar plant-pollinator data. Pollinator function has declined through time, with bees showing lower visitation rates and lower fidelity to individual plant species.

Honeybees Can't Do It Alone



Action at a Distance

Snowfall in the Sierra Nevada provides a large fraction of the water that California receives as precipitation. Knowing what factors influence the amount of snow that falls is thus critical for projecting how water availability may change in the future. Aerosols have an important effect on cloud processes and precipitation. **Creamean *et al.*** (p. 1572, published online 28 February) found that dust and biological aerosols originating from as far away as the Sahara facilitate ice nuclei formation and ice-induced precipitation in the Sierra Nevada and show how dust and biological aerosols from places as distant as Africa and Asia can influence precipitation over the western United States.

Copper in the Spotlight

Elemental copper should, in principle, be a productive catalyst for the commercial preparation of propylene oxide; however, in practice, surface oxidation under industrial conditions quickly diminishes selectivity below a useful threshold. **Marimuthu *et al.*** (p. 1590) now show that irradiating the copper with visible light during the reaction excites surface plasmon resonances that lead to reduction of the oxide coating and restore selectivity.

Making the Final Cut

Abscission, the final separation of two daughter cells, was long thought to be an unimportant step in cytokinesis, triggered merely by the

cells pulling strongly enough on the bridge to rupture it. Research over the past 10 years, however, has challenged this notion. Defects in cutting the cytokinetic bridge can lead to the formation of large networks of connected cells or to binucleate cells. **Lafaurie-Janvore *et al.*** (p. 1625) now show that the forces postmitotic cells exert on the cytokinetic bridge play an important role in abscission: Surprisingly, increasing the tension in the bridge inhibits abscission, while reducing tension induces abscission. This could provide a sensing mechanism to ensure that daughter cells establish sound connections with their surrounding cells and matrix before detaching from one another.

Lamin Loppers

The nuclear lamina provides mechanical stability to the nuclear envelope and is involved in regulation of cellular processes such as DNA replication. Defects in the nuclear lamina lead to diseases such as progeria and metabolic disorders. One of the components of the nuclear lamina, lamin A, undergoes a complex maturation process. A key player is an inner nuclear membrane zinc metalloprotease (ZMP) that is responsible for two proteolysis steps (see the Perspective by **Michaelis and Hrycyna**). **Quigley *et al.*** (p. 1604) report the crystal structure of human ZMPSTE24 and **Pryor *et al.*** (p. 1600) that of the yeast homolog Ste24p. The structures provide insight into the mechanism of catalysis and into why mutations in ZMPSTE24 lead to laminopathies.

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Balancing Humans with Apes

Shared ancestral polymorphisms between species tend to be relatively rare, and studies of trans-species polymorphisms have focused on just a few regions known for balancing selection. **Leffler *et al.*** (p. 1578, published online 14 February) performed genome-wide scans among humans and great apes and found shared polymorphisms between chimps and humans. Many of the identified variants seem to be associated with genes involved in pathogen response or defense, suggesting that this widespread balancing selection may reflect the ongoing arms race between pathogens and hosts.

Beta Bonding

Carbonyl compounds, which incorporate carbon-oxygen double bonds, are among the most productively reactive molecules in synthetic as well as biochemical contexts. The carbon directly bonded to oxygen is rendered highly electrophilic, while the adjacent (α) carbons are easily deprotonated to undergo further transformations. In contrast, the β carbons that are one step further along the chain tend to be relatively inert. **Pirnot *et al.*** (p. 1593) now show that a dual catalyst system—consisting of an amine and a photoactive metal complex—can activate the β carbons of carbonyl compounds to couple with aryl substrates.

Patchy Polar Cap

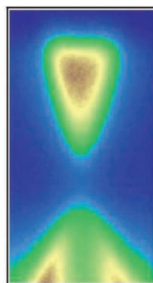
Patches of enhanced density plasma in the polar ionosphere (or polar cap patches) disturb radio communications and satellite positioning at high latitudes during magnetospheric storms. Using data from Global Positioning System satellites and a high-frequency radar network, **Q.-H. Zhang *et al.*** (p. 1597) analyzed a magnetospheric storm driven by a strong coronal mass ejection from the Sun and followed the evolution and motion of a patch of ionization throughout the polar cap. The localized dayside flow response to the solar disturbance allowed a patch to be stored and grow in the dayside polar cap, and the gaps between patches were controlled by the onset of magnetic reconnection in the magnetosphere's tail.

Fairies? No, Termites!

Fairy circles consist of circles of perennial vegetation that grow within otherwise mostly barren desert habitat on the southwest coast of Africa. Many hypotheses have been put forward to explain the creation and maintenance of fairy circles. Using long-term data collected on the distribution and physical and biological components of these features, **Juergens** (p. 1618) found that the circles are generated by the actions of the sand termite, which removes vegetation produced following intermittent rains. Once generated, the circles collect water, which sustains the growth of perennial vegetation at the edges of the circles, allowing for long-term persistence of the termites.

Magnetic Topology

Topological insulators owe their exotic properties to the peculiarities of their band structure, and one can induce a transition between a topologically trivial and non-trivial material by chemical doping. Now, **J. Zhang *et al.*** (p. 1582) have gone a step further—simultaneously observing that a magnetic quantum transition as the ratio of Se and Te is varied in $\text{Bi}_2(\text{Se}_{1-x}\text{Te}_x)_3$ thin films grown by molecular beam epitaxy and doped with magnetic Cr. Photoemission and transport experiments, as well as density functional calculations, imply that the topological transition induces magnetism.



Focusing on the Right Metabolite

A variety of human cancers, including acute leukemias and brain tumors, have mutations in the genes encoding isocitrate dehydrogenase 1 or 2 (IDH1, IDH2), which cause overproduction of a metabolite called 2-hydroxyglutarate (2HG). **Losman *et al.*** (p. 1621, published online 7 February) show that the *R*- but not the *S*-enantiomer of 2HG can transform cells and that *R*-2HG mediates transformation at least in part through effects on protein modifying EglN prolyl hydroxylases. Importantly, the transforming activity of *R*-2HG was reversible, suggesting that therapeutic strategies focusing on inhibition of *R*-2HG production or inhibition of EglN prolyl hydroxylases merit further investigation.

CREDIT: J. ZHANG ET AL.



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Grappling with Cancer

ONE OF THE GREATEST CHALLENGES IN THE STUDY OF CANCER HAS BEEN CONFRONTING THE mindset that the disease is too complex to address effectively: too many tissue types, too many etiologies, too much genetic chaos. In the 1970s, the oncogene concept provided temporary relief from this angst, because it was thought that a limited number of genes (proto-oncogenes), when activated through mutations, might turn a normal cell into a cancerous one. But what briefly seemed tidy quickly became messy again with the discovery of other classes of genes that normally protect against cancer. Most recently, advanced genome sequencing technologies have revealed a surprising fact: Every tumor contains hundreds to thousands of mutations, most of which affect only a small percentage of the cancers in any tumor type (see the Review by B. Vogelstein *et al.*, p. 1546). In addition, the high degree of cancer cell heterogeneity in each tumor suggests that we are studying a moving target that can readily dodge treatments through continual mutation. And the genetic uniqueness of every cancer raises the question of whether standardized cancer treatment protocols can ever achieve broad efficacy.

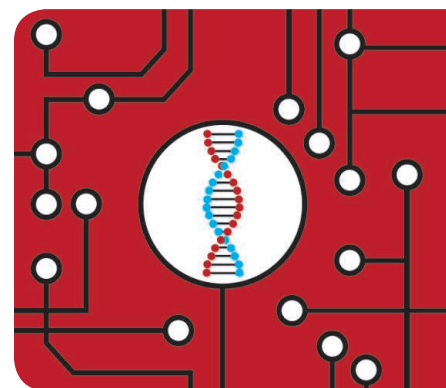
The good news is that we are now armed with dramatically more information and substantially more powerful tools to grapple with cancer's complicated nature. By necessity, past research focused on one gene at a time, and so our mindsets were necessarily restricted. Now that we can access the complete, precise genomic information about any cancer, future advances will depend on exploiting the natural genetic complexity of this disease. This will require much more than annotating lists of mutations. What is needed is the ability to detect all of the relevant components of a system and describe the complexity observed in a mathematical manner so that models can be computed. We then need to reconstruct this complexity in experimental systems and perturb those systems to test their characteristics. Ultimately, this could reveal higher-order rules that explain the possible actions of each particular cancer in terms of its gene networks.

Going forward, a transition to a systems dynamics view requires a different empiricism. A major change will be viewing each type of cancer as an experimental system by itself. Rather than analyzing many thousands of tumors somewhat superficially, we may need very deep analyses of a few well-characterized tumors, using multiple approaches for which diverse data sets can be integrated (complete DNA and RNA sequences, whole-genome epigenetic information, etc.). The tumors to be analyzed can be selected on the basis of their exceptional differences in behavior, such as sensitivity versus resistance to chemotherapy, and aggressiveness versus latency. The analyses required to validate the behavior of that cancer could be aided by using genetically engineered mouse models and patient-derived xenografts. The goal would be to generate a "whole-system" understanding of each cancer and provide a more nuanced approach to developing therapies.

But perhaps this view of each type of cancer as a unified but static unit is too simplistic. Instead, each cancer could be considered an evolutionary experiment involving a genetically plastic population of cells undergoing selection within a tumor. The genomic composition of single cells in an individual cancer before and after treatment may best uncover the genetic fluxes that lead to therapeutic resistance. Thus, a small group of individuals entering a "proof-of-concept" study could have their tumors genomically analyzed, the tumors' therapeutic sensitivity tested in patient-derived xenografts, and the *in vivo* tumor clonal response to a tailored anticancer treatment monitored by detailed analyses of circulating tumor DNA.

Which experimental approach will be the best for advancing understanding of cancer biology and improving clinical outcomes is, of course, debatable. But we are very fortunate to be in a position today to test each of the many possibilities.

— Edison T. Liu



CHEMISTRY

Making Butene in MOFs

Although homogeneous organometallic catalysts can be very efficient in solution, it can be advantageous for separations and catalyst recovery to "heterogenize" them by using linkers to attach them to a support. However, typical high-surface-area metal oxide materials used as supports for conventional heterogeneous catalysts can deactivate organometallic catalysts through unwanted interactions with the support or between nearby catalyst molecules. One alternative has been to incorporate catalysts into high-surface-area metal organic framework (MOF) materials, and Canivet *et al.* used this method with an olefin oligomerization catalyst. They started with (Fe)MIL-101-NH₂, in which trimeric iron(III) octahedral clusters are linked by 2-aminoterephthalate ligands. This MOF has the high pore volume needed to accommodate a nickel(II) catalyst as well as reactants and products. They achieved different densities of N,N-chelating centers for nickel in the MOF through reaction with 2-pyridine carboxaldehyde, which formed a methanimino bridge between some of the 2-aminoterephthalate linkers and pyridyl groups. These materials were then suspended in heptane and exposed to ~15 atm of ethylene in the presence of diethylaluminum chloride. One of them, which had a loading of 10 nickel centers per MOF cage, exhibited >95% selectivity for forming 1-butene. — PDS

J. Am. Chem. Soc. **135**, 4195 (2013).

SIGNAL TRANSDUCTION

Understanding the STIM1-ulous

The permeability of the circulatory system is carefully regulated, and disruption of the barrier can contribute to inflammation and sepsis. One important means of regulation is the action of thrombin on vascular endothelial cells. The receptor for thrombin initiates mobilization of calcium from intracellular stores, and Shinde



CLIMATE SCIENCE

Explaining Rapid Climate Fluctuations

Dansgaard-Oeschger (DO) cycles—rapid warming events with durations of approximately 1000 years, followed by a more gradual return to cold conditions—are some of the most dramatic examples of rapid climate change that occurred in the North Atlantic region over the last glacial period. Although there has been no lack of suggestions about their possible origins, all of the causal mechanisms proposed thus far have run into difficulty explaining one part of the cycle or another. Petersen *et al.* step into the fray, suggesting that DO events resulted from a combination of the effects of sea ice and ice shelves—structures that help define the margins of ice sheets—to account for both the rapid and the slower parts of the cycle. Their model relies on the ability of thin sea ice to respond quickly to changing environmental conditions and on the more gradual behavior of thick ice shelves to cause cooling. Although more work needs to be done to support this model, it is consistent with existing proxy records, model results, and modern observations. — HJS

Paleoceanography 10.1029/2012PA002364 (2013).

et al. examined the role of such signaling in control of the endothelial barrier formed by human umbilical vein endothelial cells in culture. Signals that cause emptying of calcium stores lead to calcium entry across the plasma membrane by the Orai channel protein and its calcium sensor, stromal interacting molecule 1 (STIM1). STIM1 was required for increased permeability caused by thrombin; however, the role of STIM1 required neither calcium entry nor regulation of the Orai channel. Instead, STIM1 was required to activate the small guanosine triphosphatase RhoA, which coordinates remodeling of the actin cytoskeleton and reduces cell adhesion, which leads to increased permeability. — LBR

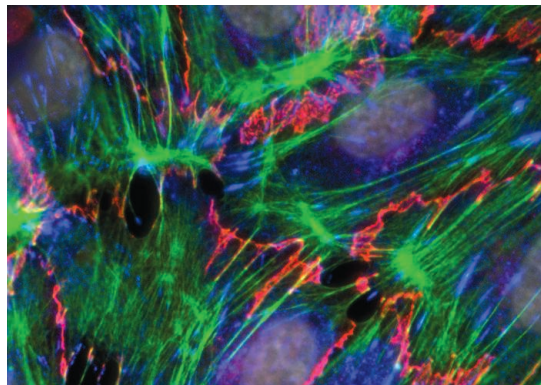
Sci. Signal. **6**, ra18 (2013).

NEUROSCIENCE

Much-Needed Neurogenesis

Destruction of nervous tissue, such as what occurs during stroke, traumatic brain injury, or neurodegeneration, usually causes deficits and malfunctions. However, brain injury can also stimulate plasticity. Perederiy *et al.* created unilateral lesions of the perforant path in the hippocampus in a mouse model and observed the response of newborn granule cells that were dividing at the time of the lesion. Using transgenic and retroviral labelling techniques, these cells could be followed and traced over the coming days and weeks. Chronic denervation triggered the proliferation of adult-generated neurons. The dendrites of these newborn

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neurons penetrated into the denervated zone but had a simpler morphology, comparable to the retraction of distal dendrites in mature granule cells. However, they could still produce new dendritic spines and could also be synaptically activated by other nonlesioned pathways. Excitatory axons from these pathways formed new synapses with newborn granule cells. Lesions are thus not only damaging: They can stimulate the production of adult-generated neurons, and these newly produced cells can show enhanced structural plasticity. — PRS

J. Neurosci. **33**, 4754 (2013).

BIOCHEMISTRY

Telomerase Times Two

In eukaryotes, chromosome ends are capped by telomeres: protein-DNA complexes that are essential for genomic stability. Telomere length is maintained by the enzyme telomerase, which comprises an RNA subunit (TER), that contains the template for synthesis of telomeric DNA repeats and a protein subunit, telomerase reverse transcriptase (TERT), that catalyzes nucleotide addition. In contrast to macromolecular machines such as the ribosome and RNA polymerase, structural information on telomerase has been limited to subdomains, and this has hindered understanding of its function. Debate continues over whether telomerase functions as a monomer or a dimer. To resolve this debate, Sauerwald *et al.* obtained sufficient quantities of purified, active human telomerase for biochemical, structural, and functional studies. Biochemical and functional data showed that telomerase functions as a dimer. It binds two telomeric DNA substrates, and two functional TERT subunits are required for activity. A structure determined by single-particle electron microscopy (EM) at better than 30 Å resolution revealed a bi-lobal architecture with a size consistent with a homodimer. Gold-labeling and fitting of a high-resolution structure of a TERT subunit into the EM density provided further insight into the architecture of telomerase. Future higher-resolution structures will probably give an even more increasingly detailed view of telomerase function. — VV

Nat. Struct. Mol. Biol. **20**, 10.1038/nsmb.2530 (2013).

PLANT SCIENCE

Fine-Tuning a Tart Grape

A good wine is a complex blend of flavors, at least some of which are based on the acidity of the wine, which is derived from the acidity of the grape. Grape berry acidity depends on



potassium concentration, with high potassium content leading to less acidic fruit. As the grape berry ripens and sugars accumulate, potassium also accumulates. Potassium physiology in grape berries, however, is not well understood. Cuéllar *et al.* have now identified in grapevine (*Vitis vinifera*) a potassium channel, encoded

by the gene *VvK1.2*, as well as kinase- and calcium-sensing partners that regulate the function of the channel. Accumulation of *VvK1.2* transcripts, which are expressed in the berry flesh, accelerated with fruit ripening. Drought stress during the berry-ripening phase

brought further increases in *VvK1.2* transcript accumulation. Increased capacity for and regulation of potassium transport may be critical for successful ripening of the grape berry as it adjusts to fluctuating conditions that result from changes in internal vascular support and external drought conditions. — PJH

Plant J. **73**, 1006 (2013).

CHEMISTRY

Ubiquitous Alkyne Activity

Over the past decade, the coupling of terminal alkynes with azides—so efficient it's been termed a "click" reaction—has become a prevalent means of linking together larger molecular fragments. One advantage of the scheme was that the alkynes appeared to be otherwise inert in cellular environments, thereby enabling high specificity in the design of various biochemical probes. Two studies now report that terminal alkynes do in fact react with certain enzymes. Ekkebus *et al.* show that appending the alkyne to the C terminus of ubiquitin leads to inhibition of deubiquitinating enzymes. Crystallography revealed covalent linkage to an active site cysteine, the product of formal Markovnikov addition of the thiol group across the carbon-carbon triple bond (though no reaction was observed with free thiol). Sommer *et al.* observed the same reaction of C-terminally propargylated small ubiquitin-like modifier (SUMO) with its protease. Scavenger experiments in both studies disfavor a radical mechanism. Both studies leveraged the observation for selective labeling of lysates and suggest that this reaction could have broad application in selective inhibition. — JSY

J. Am. Chem. Soc. **135**, 2867; *Bioorg. Med. Chem.* **21**, 10.1016/j.bmc.2013.02.039 (2013).

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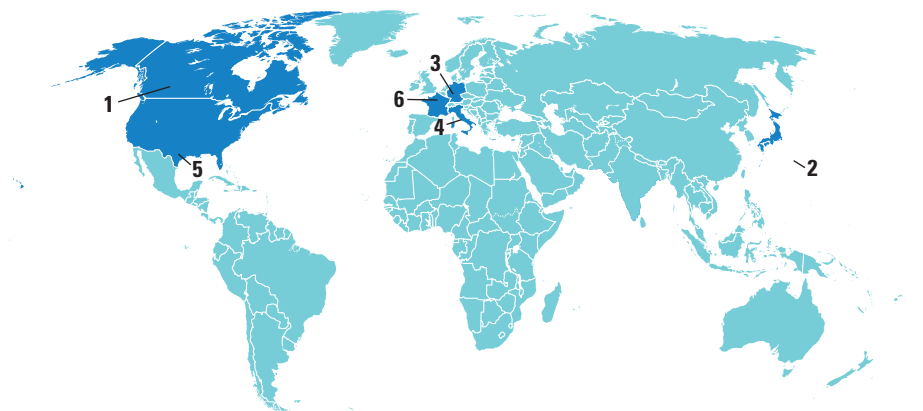


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AROUND THE WORLD



Edmonton, Canada 1

German Scientists Pull Out Of Oil Sands Project

German scientists have pulled out of a research project with Canada that sought to minimize the environmental damage caused by exploiting Alberta's oil sands. The scientists, part of the Helmholtz-Alberta Initiative (HAI), will no longer be involved in developing technologies that improve Alberta's crude oil or treat the toxic effluent from the oil sands projects.

HAI, founded in 2011, is a partnership between the Helmholtz Association and the University of Alberta. The supervisory board for one of the four Helmholtz institutes involved in the partnership voted in December to impose a moratorium on its involvement in the project, in part due to an ongoing

ban; that, along with Canada's withdrawal from the Kyoto Protocol, prompted several German politicians to ask the Helmholtz Association pointed questions about the Alberta project.

An independent assessment into HAI's environmental credentials will report its findings in June. <http://scim.ag/Alboil>

Minami Torishima, Japan 2

Japan Reports Rare Find Of Rare Earths

Japanese researchers last week reported finding high concentrations of rare-earth elements in deep-sea sediment near the island of Minami Torishima, about 1800 kilometers southeast of Tokyo.

Rare earths—such as the elements neodymium, terbium, and yttrium—are essential to a host of advanced electronics, including displays, cell phones, solar panels, and high-performance electric motors. China controls 95% or more of global production, a realization that led Japan, the United States, and other countries to hunt for new sources and develop substitutes (*Science*, 17 December 2010, p. 1598). Scientists from the University of Tokyo and the Japan Agency for Marine-Earth Science and Technology reported finding significant rare earths in seafloor sediments at 78 sites scattered around the Pacific Ocean in *Nature Geoscience* in July 2011.

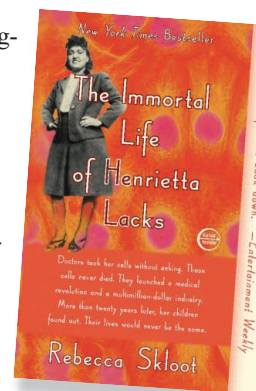
The latest find is potentially the most commercially significant, with a concentration of 6500 parts per million of rare earth elements—far higher than that in deposits found elsewhere in the Pacific or on land in China. The challenge will be recovering the ores economically; they lie in 5800-meter-deep water.

Heidelberg, Germany 3

HeLa Genome Withdrawn

Complex questions of genetic privacy have led the European Molecular Biology Laboratory (EMBL) to cut off public access to a popular cell line's genome sequence, which it had announced in a press release just 2 weeks earlier. On 11 March, EMBL researchers published a genome for the widely studied cancer cell line known as HeLa. But HeLa stands for an African-American woman by the name of Henrietta Lacks, and the research use of her cells, which were originally taken without consent, has gained publicity thanks to a popular book by science writer Rebecca Skloot. Skloot and some scientists questioned the ethics of releasing the HeLa genome, suggesting that it could reveal personal

information and violate the privacy of Lacks's descendants. "We have taken the data offline until the question has been resolved of whether the family consents to the public availability of genomic information on the cell line. ... We did not consider this an issue exactly because of the notoriety of the cells and the existence of so much molecular biological and genetic data on these long before our study," an EMBL spokesperson writes in an e-mail. <http://scim.ag/HeLaflap>



Rome 4

Stem Cell Researchers Protest Controversial Treatment

The Stamina Foundation can continue a controversial stem cell treatment in patients with neurodegenerative diseases such as spinal muscular atrophy and Parkinson's, according to a new bill signed by Italian Health Minister Renato Balduzzi on 21 March (and approved by the Council of Ministers). Over the last 3 years, a hospital in Trieste and another in Brescia have used the treatment in agreement with Stamina, although it has never been authorized by the Italian drug administration, known as AIFA.

Little is known of the treatment, which Stamina says is based on mesenchymal stem cells, and few documents related to its methodology are public, such as two pat-



A Syncrude plant in Alberta's oil sands.

debate over a possible amendment to the European Union's fuel quality directive that would restrict the use of "high-polluting" oil within Europe.

Germany (with the United Kingdom) has so far blocked the amendment, but public opposition to importing Albertan oil remains high. The Canadian government has lobbied German politicians to continue blocking the

ents issued by the United States to Stamina. After lab inspections in Brescia in 2012, AIFA called for interrupting the treatment, but patients' families appealed to courts to continue its use. Two weeks ago, Italian stem cell researchers sent an open letter to Balduzzi, asking him to bring what they called "the administration of snake oil" to a definitive end. But the new bill defines the Stamina method as a medical treatment without serious side effects and stated that it should not be stopped in patients already under Stamina's treatment.

Austin 5

Cancer Researcher Recruitment Grants Unfrozen

Texas officials will allow the state's \$3 billion cancer research agency, the Cancer Prevention and Research Institute of Texas (CPRIT), to make 25 recruitment awards that had been on hold since December. The decision is the first good news in a while for the troubled agency. Last May, chief scientific officer and Nobel Prize winner Alfred Gilman quit in protest over CPRIT's scientific review processes; two more top officials left last fall. In December, CPRIT agreed to a request from state leaders to impose a moratorium on new grants while investigations continued.

Texas research institutions, in the meantime, worried that the delay in awarding \$71.8 million in approved grants to 25 researchers from outside the state would derail the recruitments. Last week, CPRIT interim Executive Director Wayne Roberts announced that state leaders will allow CPRIT to move ahead. "We have worked hard to regain trust," and "[w]e take this action as evidence that some progress has been made," Roberts said.

Paris 6

Science Law Falls Short

After months of consultation with the French scientific community, the Council of Ministers last week adopted a draft bill for a higher education and research law. The bill consists of 20 measures that, France's science minister says, will better support students at universities and will give research a new impetus, hopefully boosting economic recovery and competitiveness. Among other changes, the bill proposes reorganizing the nation's higher education institutions into 30 or so regional groups that will collaborate with research



I See You: New Mouse Lemurs Spotted

In a century where new mammal species discoveries are rare, two new mouse lemurs made their formal debut this week. Rodin Rasoloarison from the University of Antananarivo in Madagascar came across these softball-sized primates in eastern Madagascar rain forests in 2003 and 2007. He took a small skin sample from each for DNA analysis. From afar, the gray-brown primates are virtually indistinguishable, but their genes revealed that they are two species, Rasoloarison and his colleagues reported online on 26 March in the *International Journal of Primatology*. That means there are now 20 recognized species (pictured: *Microcebus marohita*), and "I suspect there are even more mouse lemur species out there to be found," says Anne Yoder, a co-author and director of the Duke Lemur Center in Durham, North Carolina. Reduced forest cover from human activities has made mouse lemurs the most endangered mammals in the world, notes the International Union for Conservation of Nature.

laboratories and local authorities. The law also aims to help set a clearer national strategic research agenda.

But some researchers say that the bill doesn't address many of the community's concerns, including a guarantee of an adequate budget for universities and job stability for the nation's growing numbers of nonpermanent research staff members. On 21 March, as part of a call from French trade unions and research associations, students and nonpermanent scientific staff members held a protest in Paris for new permanent positions to be created. The draft bill will be debated in Parliament starting in May.

<http://scim.ag/Francebill>

NEWSMAKERS

Hopkins Biochemist to Head NIH's Basic Science Institute



Lorsch

Jon Lorsch, a biochemist at Johns Hopkins University in Baltimore, Maryland, will become director of the \$2.4 billion National Institute of General Medical Sciences (NIGMS) in August.

Lorsch, 44, uses yeast to study how cells initiate the translation of RNA into proteins. He will join NIGMS, the National

>>>

Random Sample

Atomic Science Keeps Silver Shining Bright

Armed with Q-tips, chemical coatings, and lots of elbow grease, art conservators wage unceasing war against tarnish, a thin layer of sulfide that forms on silver when it's exposed to air. Constant polishing can wear down artifacts, however. And the protective coatings now in use are notoriously uneven and last only about 20 years—a short time in the museum world.

Now, materials scientists from the University of Maryland (UMD) think they've hit upon a solution. Using an easily reversible technique called atomic layer deposition (ALD), they coated pieces of silver with layers of aluminum oxide only one atom thick. By gradually building up the number of layers, the researchers could precisely control the thickness of the film in the silver's every nook and cranny.

Ten nanometers of the ALD film could protect a silver artifact from tarnish for more than 80 years, the team reported at the March meeting of the American Physical Society in Baltimore, Maryland.

If ALD coatings could save artifacts from the damaging effects of constant rubdowns, "it's really worth the effort," says Martin Fischer, a physicist at Duke University in Durham, North Carolina, who is not involved with the project. "Once you polish off all the silver, that's it." He looks forward to future work testing the technique's effectiveness on other materials as well.

In the meantime, the UMD team is working with Baltimore's Walters Art Museum to apply the ALD film to a late 15th century Spanish cross (pictured)—the technique's first test on a true work of art.



with a monetary award of 6 million Norwegian kroner (about \$1 million). The prize honors the 19th century Norwegian mathematical prodigy Niels Henrik Abel. Deligne has previously won the Fields Medal and the Crafoord Prize, among many other honors.

FINDINGS

Gut Microbes Tied To Surgical Weight Loss

Gastric bypass surgery is an extreme remedy for obesity, but how it helps people lose weight isn't clear. After the operation, patients can't take in or absorb as many calories—but studies suggest that other things are at play, too. Work published this week in *Science Translational Medicine* now points to another possible driver: the altered balance of microbes in the gut after surgery, which can cause weight loss in animals.

The team, led by gastroenterologist Lee Kaplan and microbiologist Peter Turnbaugh of Harvard University, found that within a week of surgery, rodents showed microbial shifts in their feces similar to humans, bringing the balance closer to those of lean individuals. The group then transplanted samples from the gastrointestinal systems of mice who had and had not had gastric bypass surgery into animals raised in a germ-free environment. The treated mice lost about 5% of their body weight in 2 weeks. How gastric bypass surgery alters microbial colonies in the gut is "essentially an open question at this point," says Turnbaugh, who hopes to determine whether the findings will also translate to humans. <http://scim.ag/gastbiome>

>>NEWSMAKERS

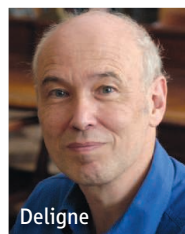
Institutes of Health's fourth largest institute, as NIH is coping with a 5% budget cut from sequestration. Lorsch says his priorities include making the case for basic research, using taxpayer funds "efficiently," and helping NIH carry out plans to improve graduate education and to attract minorities to research. Diversity in "scientific subjects, settings, and researchers," will be a theme, he says: "If we can ensure we have the most effective ways to distribute funding, it will be for the good of the nation and scientists." Lorsch replaces Jeremy Berg, who left NIGMS in July 2011.

Deligne Wins Abel Prize

Pierre Deligne has won this year's Abel Prize for outstanding work in mathematics. In its 20 March announcement, the prize committee praised his "seminal contributions to algebraic geometry and ... their transformative impact on number theory, representation theory, and related fields."

A professor emeritus at the Institute for Advanced Study in Princeton, New Jersey,

Deligne, 68, was born in Brussels. After receiving his Ph.D. from the University of Brussels in 1968, he joined the Institut des Hautes Études Scientifiques at Bures-sur-



Deligne

Yvette, France. He moved to the United States in 1984.

Deligne works in algebraic geometry, which uses multi-dimensional curves and surfaces to provide solutions to systems of equations. A versatile and creative mathematician, he has notched a string of influential proofs and forged standard mathematical tools used in several subdisciplines.

Presented by the Norwegian Academy of Science and Letters, the Abel Prize comes

Science LIVE

Join us on Thursday, 4 April, at 3 p.m. EDT for a live chat on what we've learned about dinosaurs since *Jurassic Park*. <http://scim.ag/science-live>

BY THE NUMBERS

3 Types of neutrinos indicated by the study of the cosmic microwave background by Europe's Planck spacecraft, dashing some physicists' hopes that earlier hints of a fourth neutrino would be bolstered.

£4.4 million Amount donated by an anonymous foundation to save the Royal Institution of Great Britain from eviction this month. The stay of execution gives the 200-year-old organization more time to pay off debts.

GENOME SEQUENCING

Return of Unexpected DNA Results Urged

Geneticists, ethicists, and physicians reacted with shock to recommendations released last week by the American College of Medical Genetics and Genomics (ACMG): that patients undergoing genomic sequencing should be informed whether 57 of their genes put them at risk of serious disease in the future, even if they don't want that information now. The recommendations also apply to children, whose parents would be told even if illness wouldn't strike until adulthood. The advice runs counter to the long-standing belief that patients and parents have the right to say: I don't want to know what's in my or my child's DNA.

The ACMG recommendations were a year in the making and are likely to carry substantial weight. They represent the first time that a professional society has advised labs and doctors what to do when unanticipated genetic results—called “incidental findings”—turn up in the course of sequencing a patient's genome for an unrelated medical condition.

“It will be viewed as a watershed event for the field of medical genomics,” says Leslie Biesecker, chief of the Genetic Disease Research Branch at the National Human Genome Research Institute in Bethesda, Maryland, and co-chair of the ACMG working group that wrote the report. “I think it is going to allow us to start practicing predictive medicine,” finding people at risk for cancer, life-threatening heart conditions, and other disorders years before they strike. Like his 13 ACMG co-authors, Biesecker is highly respected even by those who disagree with him and has spent years considering how to handle genetic information.

But many experts contacted by *Science*, other than those on the ACMG working group, panned the recommendations. “They say that labs and clinicians have a duty to inflict this information on patients and should warn patients before sequencing is undertaken that that's the deal,” says Susan Wolf, a

law professor specializing in bioethics at the University of Minnesota Law School in Minneapolis. Wolf is an outspoken advocate of returning incidental findings. “The fact that I support offering them,” she points out, “does not mean I support inflicting them.”

The divide reflects a tectonic shift in genetics. Sequencing of genomes and the protein-coding “exome” is becoming more and more common for patients with a range of undiagnosed disorders. No one is sure how to manage what might turn up. And there's concern that as sequencing becomes routine, it



DNA sharing. New recommendations say that labs and doctors should look for and give back specific gene data that put patients at risk of disease.

won't be feasible for health care workers to spend hours discussing cases and counseling patients, as they do today.

“We're part of medicine now,” Biesecker says. “This is something that we think a reasonable person would want to know, and, were you not to have told them, would be pretty unhappy with you afterwards.” That's why the working group didn't just recommend returning the findings that happened to surface.

They urged labs to actively look for mutations in every DNA sample sent their way.

“You can't undo the sequence,” says Robert Green, a neurologist and medical geneticist at Brigham and Women's Hospital in Boston, who co-chaired the working group with Biesecker. “To somehow mask or ignore it doesn't seem quite right either.”

When considered in the context of the roughly 21,000 genes that people carry, the list is short. It skews to cancer and includes *BRCA1* and *BRCA2*, which predispose to breast and ovarian cancer, and four genes for Lynch syndrome, which predispose to colon cancer. Also on the list are other cancer syndromes, inherited aneurysms, and cardiomyopathies. About 1% of the population would likely test positive for something. That number is certain to grow as more genes are added to the list over time.

The ACMG working group focused on mutations that significantly raise disease risk, although they note that for many of the genes, their impact varies between populations and even different families. They also limited their list to genetic findings that patients can act on to lower the chance of getting sick—via intensive surveillance, for example, or prophylactic surgery.

Under the recommendations, the only way to opt out of getting these incidental findings would be to decline sequencing for medical care altogether. “That's a draconian answer,” Wolf says. One concern of hers is that today, most of those prescribed whole genome or exome sequencing are very ill, or dealing with a very ill or disabled child. “They may be at a point in their life, or a point in their illness, where they're being showered by information,” and have difficulty taking in anything more.

David Dimmock, a medical geneticist at the Medical College of Wisconsin and Children's Hospital of Wisconsin, both in Milwaukee, has seen this firsthand. “We've had patients that said, ‘If you force us to have secondary results, we won't have our genome sequenced,’ especially among African-American patients who are more wary of this technology,” he says. Dimmock was one of 15 people whom ACMG asked to review the recommendations before their release. That said, Dimmock and his hospital do automatically

notify families of gene variants linked to a high risk of some childhood diseases for which treatment or surveillance can make a difference. They include a mutated *APC* gene, which confers an almost 100% chance of colon cancer in childhood or early adulthood.

Not only are geneticists deeply divided about the report—they can't even agree on whether it conflicts with a policy statement published in February and backed by ACMG as well as the American Academy of Pediatrics. The February report didn't directly address incidental findings in whole genome sequencing. But it argues that genetic testing for adult conditions be deferred until a child grows up. The two reports are "absolutely inconsistent," says Lainie Friedman Ross, a pediatrician and an ethicist at the University of Chicago in Illinois who helped write the February statement. Biesecker and Green disagree.

The two sides conjure up starkly different scenes in a doctor's office. Biesecker imagines a woman with a *BRCA* mutation who was never told that her DNA harbored it and a decade later turns up with metastatic cancer. But Ross worries about the flip side: creating a class of "patients in waiting" and overtreating them in ways that prove harmful.

When genetics experts were asked whether their hospitals might adhere to the new guidelines, a long pause often followed. "I think our preference would be that these recommendations be modified," was Dimmock's careful answer. "We are all extremely concerned [about] the implications this will have for our families."

Wayne Grody, the president of ACMG and a medical geneticist at the University of California, Los Angeles (UCLA), emphasizes that the guidelines are "not written in stone." But many believe that institutions may be held legally liable for not following them. Wolf notes that there is much "legal anxiety" around both returning and withholding genetic information and little case law out there as guidance.

The working group acknowledged open questions around payment, how information is returned, and genetic discrimination, among others. "They kind of don't analyze big issues that flow from" their suggestions, Wolf says.

That's true, Grody agrees. Still, "we felt almost anything was better than what had been going on. Labs, including my own at UCLA, [are] doing whole exome sequencing in the absence of any guidelines." Ethically speaking, he says, the issue "is the most difficult one I've ever dealt with in my entire career."

—JENNIFER COUZIN-FRANKEL

MILITARY RESEARCH

A Midcourse Correction For U.S. Missile Defense System

Two weeks after being sworn in as U.S. secretary of defense, Chuck Hagel made a surprise announcement on 15 March: the government would bolster its national missile defense system by buying \$1 billion worth of new equipment. The United States would install 14 large interceptor missiles at Fort Greely, Alaska, he said, augmenting the 26 already there and another four at California's Vandenberg Air Force Base. The firepower would help the United States "stay ahead" of threats from North Korea, which Hagel said is making "irresponsible and reckless provocations" by testing missiles and a nuclear device (*Science*, 22 February, p. 893).

What Hagel didn't stress is that the bulk-up of U.S.-based defenses also appears to mark the end of an even more ambitious—

this system since late 2008, he says, and "none of the tests" have been against a target with intercontinental range.

The Obama administration initially laid out the European basing plan in 2009 as concerns grew about a nuclear threat from Iran. It envisioned ultimately placing a new generation of super-high-speed interceptors on the European continent in Romania and Poland, closer to the Middle East. The aim, in antimissile jargon, was to "expand the battle space." A very fast interceptor close to the launch site would reach an attacking missile at an earlier point in its flight arc, potentially adding a first layer of defense; U.S.-based interceptors would then provide a second layer.

Planners envisioned a four-stage effort that would field successively faster standard (21-inch-diameter) Navy rockets at sea and on land; the early versions would likely be able to protect only European nations against missiles on low trajectories, but the final model—able to fly at 5.5 kilometers per second—would be able to stop even a missile on an arc from Iran to the United States.

The idea quickly drew opposition, however. The Russian government objected, saying that it posed a threat to its own strategic nuclear weapons. Some Republicans in the U.S. Congress also criticized it, saying that it diverted resources to a system not capable of intercepting long-range missiles. Last year, Representative Michael Turner (R-OH),

a member of the U.S. House of Representatives Armed Services Committee and a supporter of the U.S. missile defense program, asked for a review by Congress's investigative arm. In February, the Government Accountability Office (GAO) concluded in an unclassified summary of its report that "additional development and investment" would be needed to make it work as promised. The Obama administration, Turner charged last week, wasted time on the European scheme while cutting other projects.

GAO's findings echoed the skepticism of a review released in late 2012 by the National Academies' National Research Council. In



Missile surge. U.S. Defense Secretary Charles Hagel announces an expansion of the Alaska-based interceptor system—and cuts elsewhere.

and controversial—Obama administration plan to base interceptors in Europe that could protect the United States from a potential missile strike from nations such as Iran. As for the older U.S.-based system, critics say the Pentagon has seriously understated the time and money it will take to make it fully functional.

Although dropping the European interceptors was "the correct decision," the plan for 14 new missiles in Alaska is "deeply flawed," writes physicist Philip Coyle, a former defense adviser to presidents Barack Obama and Bill Clinton, in an e-mail. There hasn't been a successful intercept test with

an unclassified summary, the report concluded that the proposed high-speed European missile would need a diameter of greater than 21 inches—or a liquid fueled engine—to avoid “being overflowed” by an intercontinental quarry. That would require a new fleet of wider launchers. (The Navy has ruled out liquid-fuel for now on safety grounds.) The report’s authors were also concerned about damaging U.S.-Russian arms control talks. They concluded tactfully that the European system “may not be the best way” to defend the U.S. homeland.

With Hagel’s announcement, the Obama administration appears to have taken that advice to heart. It also reflects technical and political realities, Coyle says. The final European plan was “never more than a paper system, wasn’t in the cards technically, and for that reason wasn’t being funded at the requested level by Congress,” Coyle writes.

A reduced European deployment will still continue, Hagel said. But it will stop short of its original goal, instead aiming to deliver a medium-speed rocket (4 to 4.5 km/sec) within 5 years that could protect European sites. Meanwhile, the money that was to be spent on the faster European interceptors and related technology will now be used to buy 14 copies of the slower, older, and more massive interceptor fielded in the United States since 2005. The shift to these three-stage “ground-based interceptors” (GBIs), however, is rekindling questions about how well they perform.

A major challenge, Hagel confirmed at a press briefing, is improving the GBI’s targeting. “There was an issue regarding our gyro guidance system,” he said. “We certainly will not go forward with the 14 additional interceptors until we have the complete confidence that we need.”

The problem lies in a new version of a “kill vehicle,” known as CE-2, which sits atop the GBI, said Jim Miller, U.S. undersecretary of defense for policy. Twenty of the 30 GBIs in the field carry an older kill vehicle, called CE-1, which succeeded in 50% of its intercept tests. But the Pentagon declared it obsolete and switched to the CE-2. Problems with the CE-2 came to light when it failed twice in 2010 in its first intercept tests. Physicist George Lewis, a



Testing, testing. The ground-based interceptor (left), a mainstay U.S. missile defense, has not performed well in recent tests. Plans to develop a smaller, very fast (5.5 km/sec) interceptor derived from the Navy’s Aegis missile system (right) for use in Eastern Europe have been shelved.

defense critic at Cornell University’s Judith Reppy Institute for Peace and Conflict Studies, thinks it is a “deep” design flaw, possibly a failure caused by high-frequency vibrations that occur during flight. For the moment, the government has stopped CE-2 production while the problem is diagnosed. No new intercept test has been scheduled yet, Miller said, adding: “We’ll be looking to try to do this within this calendar year.”

Fixing the CE-2, however, may not be enough to improve the GBI system, other critics argue. Coyle, for instance, notes that the same National Academies’ study that dinged the European plan also found that the U.S.-based GBI network “lacks fundamental features long known to maximize” effectiveness against “even limited threats.” That review suggested that the government start over, build an entirely new interceptor, and eventually use current GBIs as test targets.

Stepping up the pace and scope of testing will be an urgent issue for the U.S. Missile Defense Agency (MDA), which runs the program. But testing is expensive. Lewis figures that each full-scale intercept test costs “\$200 to \$300 million, conservatively.” (MDA’s 2013 budget is more than \$7 billion of a total missile defense budget of about \$9 billion.) For an agency whose task is partly to build an image of invulnerability, poor test results can be devastating. And some analysts fear that budget reductions imposed by the automatic cuts known as the sequester could tip incentives toward even less frequent testing.

Money problems may also sharply limit other proposed options for improving U.S. missile defenses in the next few years. While MDA is buying, testing, and deploying 14 new GBIs, it may also be asked to build an entire new interceptor launch base in the United States. The academies’ report, for example, suggested building a second ground-based interceptor site in upstate New York or northern New England. This would expand the ability to target incoming missiles in the “midcourse” stage of flight—the portion that GBIs are meant to cover—according to the report.

Representative Turner and other Republicans are pushing for an East Coast base as well; they helped get startup funds through Congress last year, authorizing \$100 million to scope out environmental impacts at several locations. Planners are eyeing areas in Caribou, Maine, and Fort Drum, New York.

Coyle warns that the budget impact of moving ahead on an East Coast site would be “enormous.” Extrapolating from similar projects he’s seen, he estimates the annual cost would be “billions of dollars.” The money would be far better spent, he says, working on a fundamental technical problem that hasn’t been solved—getting an interceptor to target real warheads reliably and not get fooled by debris and decoys.

The “Star Wars” battles that raged during the Reagan administration 3 decades ago appear to be headed for a rerun.

—ELIOT MARSHALL

U.S. BUDGET

Congress Limits NSF Funding for Political Science

"How often can you trust the government to do what is right?"

That question appears on the American National Election Studies survey, which has probed the attitudes of U.S. voters on a range of topical issues just before and immediately after every presidential election since the 1950s. U.S. scientists are starting to ask themselves the same question after Congress inserted language into the final 2013 spending bill (see next page) last week that jeopardizes the future of the election survey and other political science research funded by the National Science Foundation (NSF).

The language, the handiwork of Senator Tom Coburn (R-OK), prohibits NSF from spending any money on political science "except for research projects that the [NSF director] certifies as promoting national security or the economic interests of the United States." Passed by voice vote in the last hours of a week-long debate on the spending bill, the amendment is the first time such an attack on the discipline has cleared both houses of Congress. On 26 March, it became the law of the land.

"Political science is a subject that doesn't merit the same investment from taxpayers as the hard sciences that will further the nation's competitiveness and economic development," says Aaron Fobes, a spokesman for Coburn.

"Studying the Senate filibuster [a reference to a previously funded NSF award] does nothing to help a veteran who has lost a limb or to find ways to protect the nation's computer networks." Fobes says that any certifications, to be posted on NSF's Web site, will allow the public to hear NSF's rationale for funding any particular proposal.

The language puts NSF, which provides 95% of the government's overall support for academic political science, in a difficult position. Should it continue with business as usual for the \$11 million program and risk antagonizing an influential legislator? Or should it fund only those projects that clearly meet the criteria Coburn laid down, on the assumption that Coburn is hoping NSF will shut down the program?

It's a pressing issue: One of two yearly review panels is scheduled to meet in May to pass judgment on roughly 100 research proposals, with awards to be made over the summer. (Success rates are typically 15% to 20%.) The next deadline for submissions is mid-August, after which the review process begins anew. Coburn's amendment would seem to support only a narrow swathe of subjects that researchers want to study, although some political scientists say that nearly everything they do ultimately enhances the nation's economic well-being and its national security.



Persistent. Senator Tom Coburn (R-OK), shown at a 2009 hearing, has long questioned the value of federally funded political science research.

NSF is just beginning to address these questions, says Myron Gutmann, head of the social, behavioral, and economic sciences directorate, the parent unit for the political science program. "The NSF director has asked us and the Office of [the] General Counsel to examine the legislation and provide options about how we might better manage our programs under the terms of this legislation," Gutmann says. "But we haven't done that yet."

Gutmann notes that "NSF's charter is to fund basic research across all disciplines. And my starting point is to honor that charter with respect to how we manage our programs." Asked for a timetable for the review, Gutmann says, "We're going to do it right. ... We will have an answer in time to make good

decisions on the proposals that are pending."

Many scientists believe that Coburn's amendment could undermine the entire U.S. research enterprise if it enshrines the idea that Congress can do a better job of selecting the most deserving research proposals than a panel of experts in a particular field. "The American research enterprise has flourished precisely because of this Nation's commitment to independent peer-review of grant proposals, without political interference," says John Holdren, the president's science adviser, in a statement to *Science*. "Legislatively imposed restrictions on an entire class of research do not serve the national interest."

Former NSF Director Neal Lane, a Democrat, calls the amendment "an assault on all science" and urges the agency "not to give an inch." Lane, a physicist who served as President Bill Clinton's science adviser after 5 years as NSF director, asserts that "many Republicans want to kill any science they can get their hands on because it produces results that they don't want to hear."

Criticism of Coburn's amendment crosses party lines. "The idea of labeling hard and soft sciences doesn't make any sense to me," says Arden Bement, appointed by Republican President George W. Bush first to lead the

National Institute of Standards and Technology and then NSF. "Some of the hardest science is done by the so-called soft sciences," says Bement, a materials scientist whose career spans academia, industry, and government. "The real issue is complexity, and human beings are the most complex systems in the world."

Bement also throws in Coburn's face the frequent criticism from conservative legislators that Democratic administrations are trying to "pick winners and losers" and that the free market should determine which research is worthy of support. "It strikes me as a huge dichotomy that the same members of Congress who are so adamant against picking winners and losers have done exactly that in this case," says Bement, who left NSF in

2010 to become director of the Global Policy Research Institute at Purdue University.

The American National Election Studies (electionstudies.org) runs one of two large ongoing studies funded by NSF's political science program. (The General Social Survey, run by the University of Chicago, is the other.) The election survey was first fielded in 1952 and has been copied by dozens of nations since then, says Vincent Hutchings of the University of Michigan. He's a principal investigator for the survey, done jointly with Stanford University and currently funded by a 5-year, \$10 million NSF grant that runs through 2014. "It's become the gold standard for understanding how your democracy works," Hutchings says.

The survey's most valuable element, he notes, is its long time series. "That stability allows us to ask the same question to a national representative sample—like voters' trust in government—and understand whether it has gone up or down over decades."

The hourlong survey, conducted mostly in person, has helped scientists understand basic concepts such as party identification and its relationship to voter turnout. And while Hutchings says the survey's scientific rigor has been the litmus test for ongoing NSF support (he's awaiting a call for proposals this spring to support work on the 2016 elections), he also believes that it could meet Coburn's narrow definition. "How a country thinks about its political system is certainly a component of national security," he says.

The amendment technically applies only to NSF grants made during the rest of the 2013 fiscal year, which ends on 30 September. But unless Congress passes NSF's 2014 spending bill on time, a vanishingly rare occurrence these days, the guidelines would extend into the new fiscal year. (After that, Congress would have to renew it annually.)

Jim Granato, who co-managed NSF's political science program in the early 2000s and is now director of the Hobby Center for Public Policy at the University of Houston in Texas, thinks that Coburn may achieve his goal in the short run. "I think people will be risk-averse," says Granato, who has a proposal pending before the spring panel. "Writing a proposal takes a lot of time, and NSF already has two criteria that proposals must meet—intellectual merit and broader impacts. I expect what they'll do is call up NSF and ask [program officer] Brian [Humes] what they should do."

"I feel sorry for Brian," Granato adds. "It's going to be total chaos."

—JEFFREY MERVIS



A little help ... Senator Mikulski, seen here visiting a NASA facility in her state, provided some relief for the agency.

U.S. BUDGET

U.S. Science Agencies Finally Have (Reduced) Budgets for This Year

U.S. research agencies finally know their budgets for the rest of the fiscal year after Congress completed work last week on a bill to fund the government through 30 September. Although the Senate managed to reduce the pain for some programs, only one has more to spend than in 2012.

Agency heads are now in the process of finalizing 2013 spending plans, which must then be approved by Congress. A few agencies, notably the National Science Foundation (NSF) and NASA, received more money—and were given more flexibility—than they would have had under the spending bill approved by the House of Representatives. (The bill is known as a continuing resolution [CR] because it basically freezes agency budgets at 2012 levels.)

However, no agency has been spared the \$85 billion, across-the-board reduction known as sequestration that went into effect on 1 March. So every civilian agency, even the "winners," must subtract 5% from whatever Congress has now given them, a cut that is effectively 9% because the reduction must be absorbed with only 7 months left in the fiscal year.

Confused? You're not alone. Sequestration, the first year of a decadelong process to slice \$1.2 trillion from the federal budget, wasn't supposed to happen because both parties agreed that its mandatory provisions were "stupid," if not "devastating." But the Republican-led House inserted it into its version of the \$984 billion CR and passed it on 6 March. The Senate, despite its Democratic majority, accepted the House approach and instead made some tweaks at the margins before passing its bill on 20 March. Less than 24 hours later, the House signed off on the Senate version and then recessed for a 2-week Easter break.

The Senate pulled NSF, NASA, and six Cabinet departments out of the CR and gave them a regular appropriations bill, thanks to an agreement between the chair of the appropriations committee, Senator Barbara Mikulski (D-MD), and its ranking member, Senator Richard Shelby (R-AL). That agreement, for example, allowed Mikulski to increase the appropriation for several NSF programs beyond what they would have received under the CR and offset more than half of the \$356 million sequester, bringing NSF's overall 2013 budget to \$6.88 billion. Unfortunately, that's still \$150 million below its 2012 level.

Similarly, NASA received \$45 million more for planetary sciences than it would have gotten under the CR. That's a boon for further Mars exploration and for planning a possible visit to Jupiter's moon Europa. In the one exception, the National Oceanic and Atmospheric Administration (NOAA) will get a boost of about \$300 million, with funding from a Superstorm Sandy relief bill approved earlier this year offsetting the impact of the sequester. The final legislation also gives NOAA greater budget flexibility in building new weather satellites and upgrading research aircraft.

In contrast, the National Institutes of Health remains under the continuing resolution. Legislators tossed it a \$67 million crumb, but that's hardly enough to ease the pain of the \$1.5 billion sequester for researchers whose work is dependent on the \$31 billion agency.

More details from the final 2013 spending bill are available at <http://scim.ag/CR-2013>. And there's no rest for the budget-weary: The Obama administration's wish list for science will be included in the president's 2014 budget submission to Congress, due out the week of 8 April.

—JEFFREY MERVIS

COSMOLOGY

Universe's High-Def Baby Picture Confirms Standard Theory

The news sometimes lies in what scientists don't say. Last week, researchers released the best image yet of the mottled afterglow of the big bang—the cosmic microwave background (CMB). Data from the European Space Agency's (ESA's) Planck spacecraft confirm cosmologists' standard model of how the universe was born and what it consists of. But some scientists had hoped that Planck would reveal some conceptual corner where the standard model doesn't quite work and provide a clue to a deeper understanding. Planck researchers reported no such thing.

Some cosmologists worry that they may be stuck with a theory that explains every measurement but leaves larger questions unanswered. Such a stalemate has stymied particle physicists for 30 years, as their own standard model accounts for everything discovered with atom smashers—including the recently

the galaxies. They also left tiny variations in the temperature of the CMB across the sky, which Planck, launched in 2009, has mapped to great precision.

From studies of the CMB and other measurements, cosmologists can deduce the composition of the universe. Planck refines those measurements, particularly those of NASA's Wilkinson Microwave Anisotropy Probe (WMAP), which collected data from 2001 to 2010 (*Science*, 14 February 2003, p. 991). According to Planck, the universe consists of 4.9% ordinary matter, 26.8% mysterious dark matter whose gravity holds the galaxies together, and 68.3% weird, space-stretching dark energy. It is 13.8 billion years old, 100 million years older than WMAP found. The standard cosmology fit the key distribution of the sizes of superimposed hot and cold spots almost perfectly,

Efstathiou reported no such deviations from the standard model. "The data don't want any of these things," he said.

The data do show a few peculiarities. On large angular scales, some "anomalies" appear, Efstathiou says. For example, the northern half of the sky shows weaker temperature variations than the southern half. Such oddities had been seen by WMAP, and they could signal details of how inflation began, Tegmark says. "The universe may be trying to tell us something," he says.

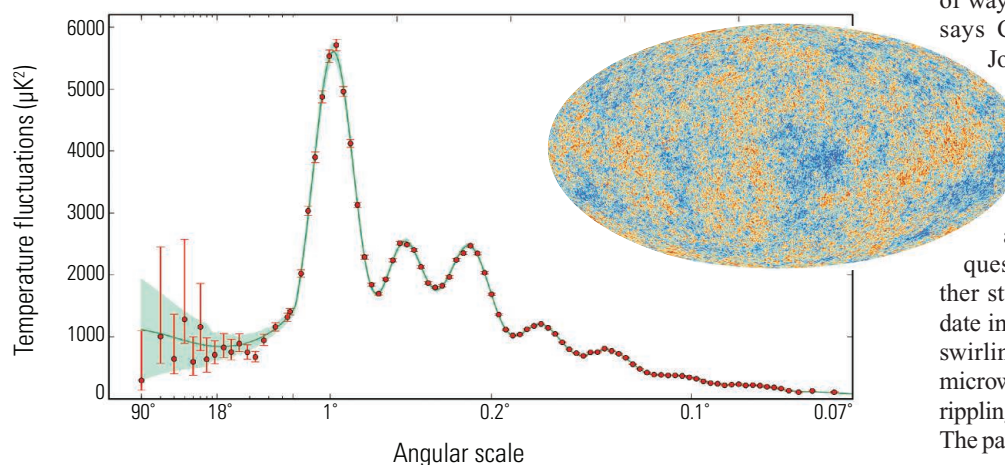
Interpreting such oddities is dicey, however. The ripples in the CMB emerged from a random process. So chance alone should produce some suggestive but meaningless patterns, just as the sheer variety of clouds guarantees that you can always find one that looks like some sort of animal. "If you have a rich data set that you can rearrange in a lot of ways, you'll eventually find something," says Charles Bennett, a cosmologist at Johns Hopkins University in Baltimore, Maryland, who led the WMAP team.

Many cosmologists say their field still has a bright future. The standard theory doesn't specify what dark matter and dark energy are or how inflation worked. So major questions remain to be answered. Further studies of the CMB could soon elucidate inflation. Researchers are searching for swirling patterns in the polarization of the microwaves, which stem from gravity waves rippling through the universe during inflation. The patterns would let scientists peek into that process, Bennett says.

But if such discoveries don't come, then cosmologists may find themselves straitjacketed by their less-than-satisfying standard model. Such an eventuality has been foreseen. In 1996, John Horgan, a science journalist now at Stevens Institute of Technology in Hoboken, New Jersey, argued in his book *The End of Science* that cosmologists and particle physicists were reaching the limits of what they could discover. Scientists ridiculed Horgan, and 2 years later his prediction seemed daft when astronomers discovered that the expansion of the universe is accelerating, revealing the first evidence for dark energy.

Now, dark energy is part of cosmology's standard model, which suddenly appears much more rigid. "Scientists will try to put the best spin on it, but they must be disappointed," Horgan says. "I hate to say I told you so, but I'll say it anyway."

—ADRIAN CHO



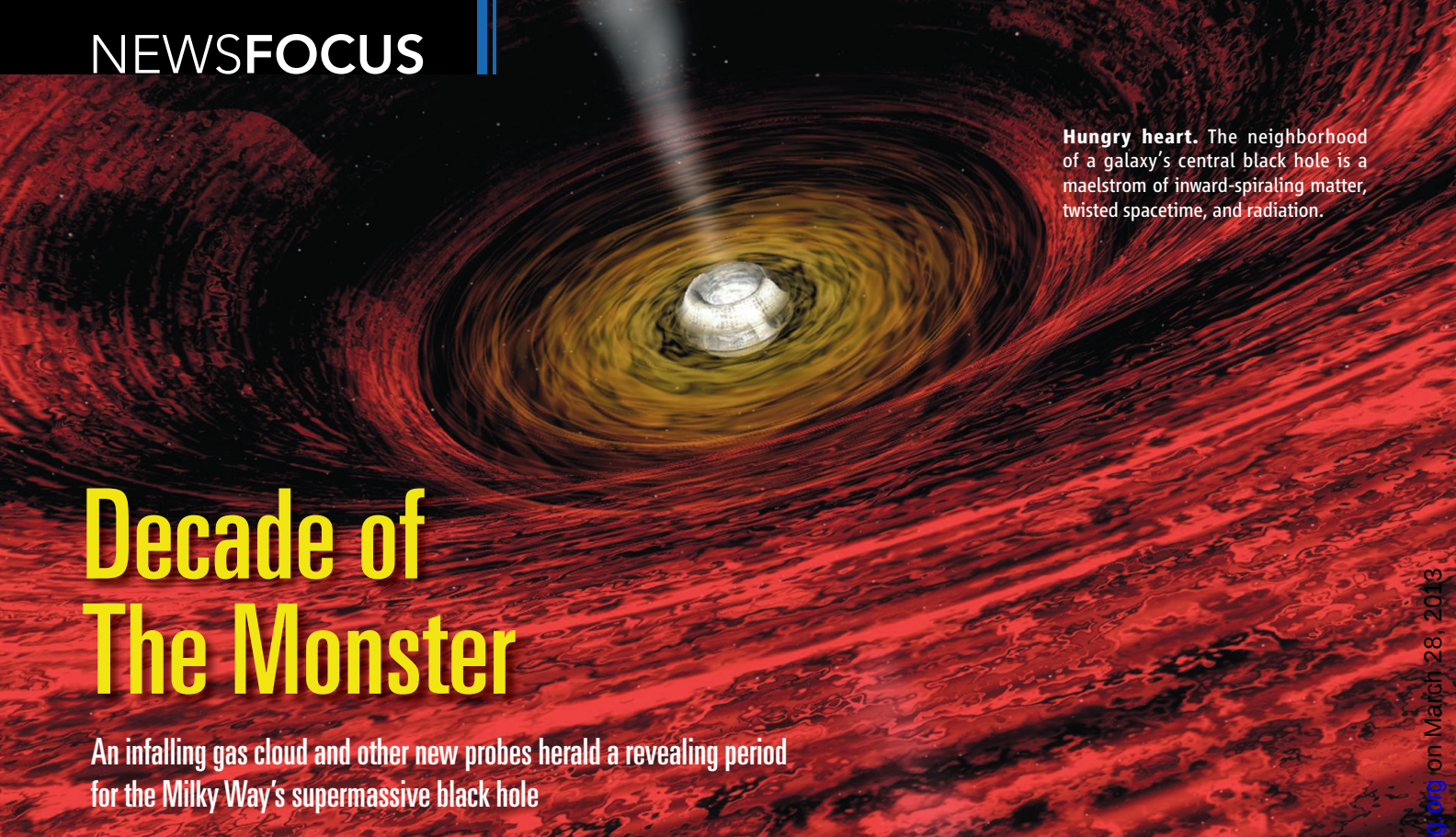
Like a glove. The six-parameter standard model fits distribution of different-sized temperature ripples in the cosmic microwave background (*inset*) almost perfectly.

spotted Higgs boson—but leaves bigger conceptual questions untouched. "The tyranny of the standard model, which has plagued particle physics for decades, has now reached cosmology as well," says Max Tegmark, a cosmologist at the Massachusetts Institute of Technology in Cambridge.

According to cosmology's standard model, the universe sprang into existence in the big bang as a hot, dense soup of matter and energy. In the first 10^{-30} seconds, space itself expanded faster than light speed. Such "inflation" pulled space geometrically "flat" like a taut bedsheet. Simultaneously, it magnified tiny quantum fluctuations in the density of the primordial soup. Those fluctuations eventually seeded the formation of

reported George Efstathiou, a cosmologist at the University of Cambridge in the United Kingdom and a Planck team member, at a 21 March press briefing at ESA headquarters in Paris (see figure, above). That's astounding, given that the ad hoc theory has only six adjustable parameters.

More important is what Efstathiou didn't say. Scientists had a short list of things that they hoped Planck would find. For example, the temperature variations in the CMB might have been not quite random, or the inferred distribution of density fluctuations might have lacked a particular statistical characteristic. Either observation would have ruled out the simplest models of inflation, in which a single "quantum field" does the inflating. But



Hungry heart. The neighborhood of a galaxy's central black hole is a maelstrom of inward-spiraling matter, twisted spacetime, and radiation.

Decade of The Monster

An infalling gas cloud and other new probes herald a revealing period for the Milky Way's supermassive black hole

IT COULD BE THE MOST PHOTOGRAPHED snack in the history of the galaxy. Either late this year or early next, the giant black hole at the center of the Milky Way will devour a blob of gas hurtling toward it at more than 2000 kilometers per second.

A vast array of telescopes in space and on the ground is poised to record the feast, which could rouse the Milky Way's gravitational monster from an extended period of dormancy—and reveal why the massive body appears to have been on a near-starvation diet for centuries. The new details may also provide insight about the puzzling dining habits of similar supermassive black holes believed to lie at the core of nearly every heavyweight galaxy.

But before astronomers can attempt to answer these questions, they must nail down several basic properties about the parcel of gas known as G2: its origin, mass, and orbit. Scientists are not even sure when the gas will pass closest to the Milky Way's 4-million-solar-mass central black hole, known as Sagittarius A* (pronounced "A-star").

G2's encounter with the black hole is just the beginning of what is shaping up to be a decadelong effort to unlock the secrets held by Sagittarius A*. Because its enormous gravitational pull traps light, astronomers can never see the heart of the galaxy directly. But they can glean information about its spin, mass, and size by studying the faint flickers

of radiation emanating from surrounding gas and dust as they heat up and spiral into the invisible beast.

Now, researchers are assembling a sensitive array of radio telescopes to perform a new trick: recording the black hole's shadow. And a star set to make its closest approach to Sagittarius A* in 2018 promises to probe in unprecedented detail the predicted curvature of spacetime just outside the body, testing Einstein's general theory of relativity in a gravitational regime more extreme than has ever been possible.

Researchers initially predicted that G2 would make its fatal rendezvous in June, coming closer to Sagittarius A* than a distance seven times that between the planet Neptune and our sun. Additional data shifted that prediction to September. Now, a new analysis by Andrea Ghez of the University of California, Los Angeles (UCLA), and her colleagues—one of two teams that have monitored the motion of stars at the galactic center for some 20 years—suggests that closest approach might not happen until March 2014. Ghez presented the findings on 14 March at a seminar celebrating the 20th anniversary of the Keck Observatory in Hawaii.

Online
sciencemag.org
Podcast interview
with author Ron
Cowen (http://scim.ag/pod_6127).

"The real question is, what is G2 and how much mass it is really going to dump onto the black hole?" Ghez says.

That uncertainty may seem surprising given that G2 has been closely monitored with two of the largest telescopes on Earth since its discovery in 2011 by Stefan Gillessen of the Max Planck Institute for Extraterrestrial Physics in Garching, Germany, and his colleagues. G2, however, is a faint, infrared-emitting source located in the most congested region of the galaxy, the packed metropolis of stars and clumps of gas orbiting Sagittarius A*. As a result, Ghez says, "these are very, very difficult observations to make."

At first, Gillessen and his co-discoverers thought that G2 was a lone gas cloud with the mass of three Earths. In the 11 September 2012 issue of *Nature Communications*, Abraham Loeb and Ruth Murray-Clay of Harvard University suggested that G2 could be a disk of gas orbiting a star, similar to the circumstellar gas disks that give birth to planets. Material boiled off the disk by ultraviolet radiation from other stars and then elongated by the black hole's tidal gravitational forces could produce a gas cloud, including a tail like the one astronomers have observed.

Whether lone cloud or stellar shell, G2 ought to brighten as it heads closer to the galactic center, where intense ultraviolet radiation from closely packed stars should ionize its hydrogen gas and set it aglow in

the near infrared. However, archival images recorded between 2008 and 2012 showed that G2 maintained an essentially uniform brightness, Gillessen and his colleagues reported on 1 February in *The Astrophysical Journal*.

The discrepancy prompted Nick Scoville of the California Institute of Technology in Pasadena and Andreas Burkert of the Max Planck Institute for Extraterrestrial Physics and the University Observatory Munich to propose a new model. In a paper posted on the arXiv preprint server on 26 February (<http://arxiv.org/abs/1302.6591>), they suggest the gas is a steady wind blown out by a young star and that radiation from the star itself, not from its neighbors, ionizes the gas. Those properties explain G2's unchanging brightness as it heads ever closer to Sagittarius A*, the team says.

If G2 is held together by the gravity of a star, less material will detach at closest approach and fall onto Sagittarius A*. That could mean fewer fireworks during this go-round, although the star's gravity might keep the cloud intact for several more passages around the black hole, giving it additional opportunities to feed and revive the quiescent beast.

Exactly what astronomers will see also depends on just when the cloud arrives at Sagittarius A*. In some ways, later is better. Telescopes on Earth can't see the Milky Way's center between October and February, when our planet arcs through the part of its orbit that places Sagittarius A* on the far side of the sun. Orbiting telescopes, including NASA's Chandra X-ray Observatory, have a narrower blackout window: between November and January. G2 watchers will be disappointed if anything exciting happens during those times.

Tracking the fireworks

Regardless of when G2 makes its closest approach, the light show is likely to unfold in three stages, Loeb says.

The first fireworks could erupt just as the gas makes its closest approach to the black hole, when the tidal gravitational forces from Sagittarius A* shred the gas into spaghetti-

like filaments or droplets. X-ray emission will flare up if G2 continues to move at supersonic speed, producing a bow shock wave as it plows into the denser gas near the black hole, and should peak at closest approach, Loeb says.

The next phase could start a month or two

plasma far off in space—critical information for understanding the mechanism by which gas falls into and fuels a black hole.

Once the gas gets dumped onto the accretion disk, it could take several months to several decades for the remains of G2 to complete

its death march, spiraling downward through the disk and disappearing forever inside the black hole's event horizon. A silent scream of radiation at all wavelengths, including x-rays, infrared light, and radio waves, may accompany its demise, although Loeb says that it may be hard to distinguish from emissions due to other processes that feed the black hole.

Several astronomers are worried that even the short-term effects of G2 may be

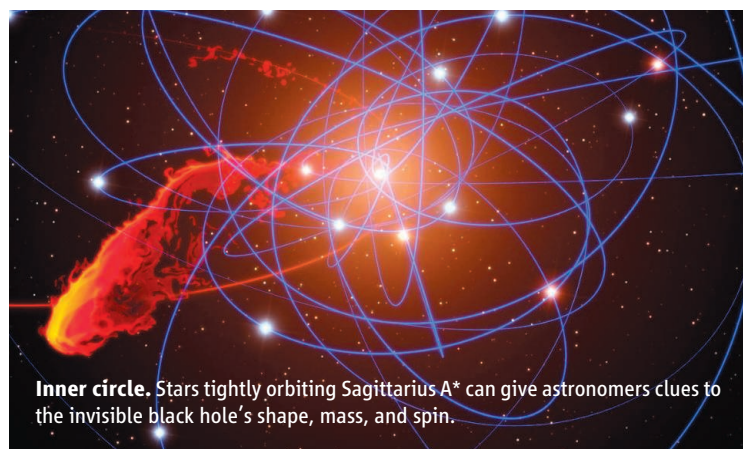
tricky to tease out from the normal variability of Sagittarius A*. Though unusually dim, the black hole's accretion disk can sometimes generate a 10-fold increase in infrared light on a timescale of minutes. That flash of light is similar to the predictions of what G2's infall might produce. How can astronomers disentangle the impact of the gas cloud from the black hole's intrinsic fluctuations?

Leo Meyer, a member of Ghez's team, began pondering that question last spring while working with UCLA finance professor Francis Longstaff on methods to predict the behavior of Sagittarius A* using the same

kind of time-series analyses used to forecast stock market volatility (see figure, left). "The big question now," Meyer says, "is whether the passage of G2 will lead to something like a stock market crash": a major change in the behavior of Sagittarius A* from an unusually dim bulb to a much more voracious, glowing black hole.

Until recently, astronomers were afraid that the black hole's natural

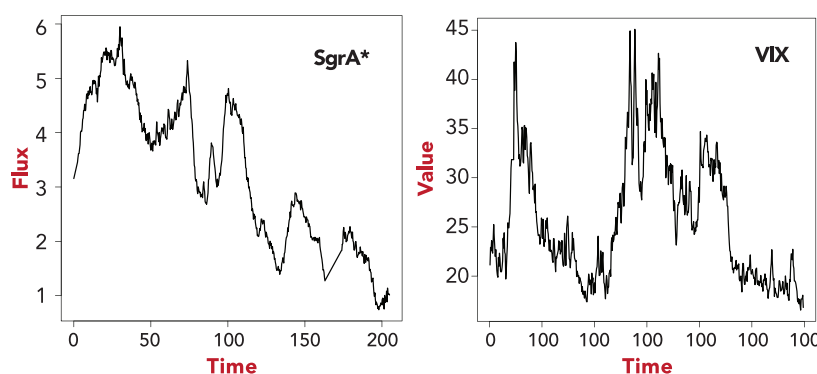
mood swings might make such a transition hard to spot. Research had indicated that Sagittarius A* flipped between two distinct states: a quiescent low state and a high state marked by sharp outbursts of fluctuation. Such bipolar behavior could make it difficult to discern any brightening due to the impact of G2. But after a closer look at the



Inner circle. Stars tightly orbiting Sagittarius A* can give astronomers clues to the invisible black hole's shape, mass, and spin.

later, when the shredded gas dives inward and strikes a proposed structure called the accretion disk—the swirling doughnut of matter believed to surround and feed the black hole. If the cool gas from G2 slams onto the warm disk, it could generate both x-rays and radio waves. Just how much radiation will be produced depends on the density, temperature, and extent of the disk—all unknown quantities at the moment, Loeb notes. "If we detect this brightening, we could constrain the unknown properties of the [accretion] disk for the first time," he says.

Timing how long the gas takes to travel



Bullish. UCLA researchers are modeling fluctuations in the infrared brightness of Sagittarius A* (left) using methods developed for studying the Standard & Poor's 500 stock market index (right).

to the disk may also shed light on conditions near the galactic center, says Avery Broderick of the Perimeter Institute for Theoretical Physics in Waterloo, Canada. By studying how G2 gives up its angular momentum to other gas parcels there, Broderick says, astrophysicists may be able to infer for the first time the viscosity of a gas or

data, Meyer and Longstaff concluded that the “low state” readings were instrumental noise from the Keck telescope and that Sagittarius A* was simpler—although more variable—than astronomers had believed. Now, Meyer says he’s optimistic that after only a few nights of timely observations at Keck, he and Longstaff will know whether and by how much G2 has boosted the output of Sagittarius A* and shifted the black hole into a new, more active regime.

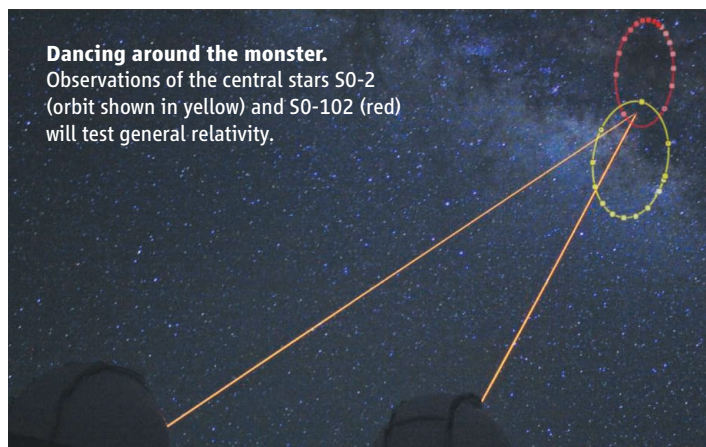
New horizons

Even after G2 passes by and hoopla about this gas cloud dies down, aficionados of the galactic center will have a lot to look forward during the rest of the decade.

Two years from now, if all goes according to plan, a network of four radio telescopes known as the Event Horizon Telescope, which has already revealed new insights about the properties of Sagittarius A* and the giant black hole at the center of the nearby galaxy M87, will greatly expand its own horizons (*Science*, 27 January 2012, p. 391). Twenty or so radio dishes from the Atacama Large Millimeter/submillimeter Array (ALMA), the radio array now nearing completion in Chile’s Atacama Desert, are scheduled to join the network in 2015 along with the 10-meter South Pole Telescope. Working in tandem, ALMA and the other radio dishes will double the resolution of the Event Horizon Telescope, creating a virtual Earth-sized radio telescope powerful enough to make landmark observations of Sagittarius A* and its accretion disk, says the telescope’s coordinator, Sheperd Doleman of the Massachusetts Institute of Technology Haystack Observatory in Westford, Massachusetts, and the Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts.

The boost in resolution will enable the telescope to make actual images of the region surrounding Sagittarius A* and hunt for a predicted feature known as the black hole’s

shadow. Although nothing, not even light, can escape a black hole’s grasp, matter that gets pulled into the monster gets crushed by the extreme gravity and heats up to billions of degrees, illuminating the region around the black hole. Most of the radiation falls into



Sagittarius A*; the light that just misses getting trapped is bent by the monster’s gravity into a thin ring or halo that frames the black hole’s shadow (see figure, below). Deviations from the halo geometry could indicate that Einstein’s theory doesn’t accurately describe the way gravity curves or distorts spacetime near a black hole and that the theory might need to be revised.

Objects swooping past Sagittarius A* may provide other important clues. In 2018, a bright star known as S0-2, discovered nearly 2 decades ago, will put Einstein’s the-

Spectra of the star will reveal the gravitational redshift of the starlight—the amount by which the mass of Sagittarius A* has curved spacetime at the galactic center. The observed redshift can be directly compared with the amount that general relativity predicts.

By precisely monitoring the 3D motion of the star, which circles the black hole about every 16 years, Ghez’s team hopes to determine whether the star’s closest approach to Sagittarius A* occurs at the same place in its orbit or whether it slowly moves or precesses about the supermassive black hole under the sway of the invisible body’s extreme gravity.

The precession is a much smaller fingerprint than gravitational redshift and is much more difficult to measure because of all the other objects—countless stars and clumps of gas—tugging on S0-2 at the Milky Way’s crowded center. “If we had a pure system of a black hole and one star, we wouldn’t be worried, but the center of the galaxy is simply a mess,” Ghez says.

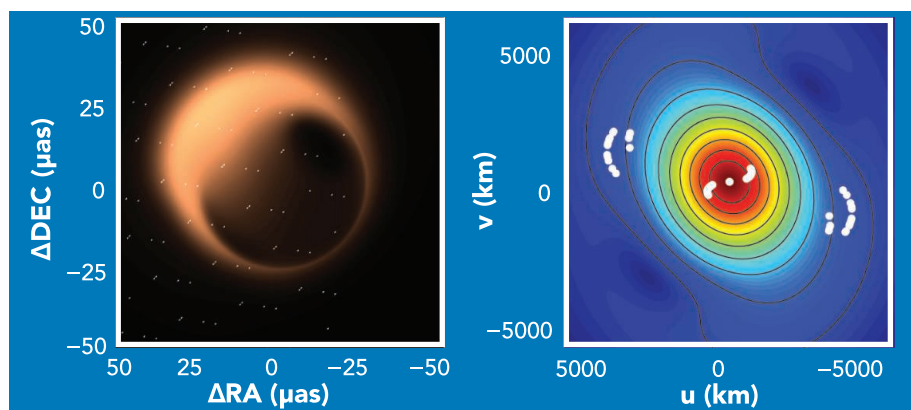
Fortunately, her team recently discovered another central star with a shorter orbit of 11.5 years, Ghez and her colleagues reported in the 5 October 2012 issue of *Science*. Although the star is only one-sixteenth as bright as S0-2, its presence will help distinguish gravitational perturbations on the brighter star’s orbit due to Sagittarius A* from those exerted by other stars and gas at the galactic core.

In 1919, astronomers measuring how stars appeared to change position as the sun’s gravity bent the path of their light made Einstein a celebrity and transported his theory to the forefront of public imagination. Nearly a century

later, a star 26,000 light-years from Earth promises to test Einstein on a far grander scale and determine if the weirdest object in the universe—a supermassive black hole—can alter the fabric of spacetime.

—RON COWEN

Ron Cowen is a freelance writer in Silver Spring, Maryland.



Shadow play. Simulation (left) shows the arc of light and the shadow formed by hot material near Sagittarius A*. At right, measurements by the Event Horizon Telescope (white spots) are overlaid on simulated data.

ory through its paces when it comes within four times the Neptune-sun distance of Sagittarius A*—about half as far as G2’s closest approach (see image, top). Two fingerprints of the star—the light it emits and the motion of the star through space—will test relativity in different ways, Ghez notes.



Survivors. This coral-mangrove ecosystem in the U.S. Virgin Islands is thriving despite ocean conditions that killed nearby reefs.

MARINE CONSERVATION

As Threats to Corals Grow, Hints of Resilience Emerge

Some reefs are showing a surprising ability to resist or bounce back from damage. Could such resilience help corals survive in a rapidly changing ocean?

Eight years ago, a blistering heat wave sent local sea temperatures soaring in the eastern Caribbean, killing more than one-half of the region's coral reefs. Many have yet to recover. But in Hurricane Hole, a sheltered bay off St. John in the U.S. Virgin Islands, one vibrant coral ecosystem survived unscathed. "We've identified more than 30 coral species" that avoided the catastrophe, says Caroline Rogers, a marine biologist with the U.S. Geological Survey (USGS) in St. John. "The diversity is astounding."

Rogers has been trying to understand what made the corals in Hurricane Hole so resilient, and she has plenty of company. Around the globe, a growing corps of scientists is searching for resilient reefs and then trying to identify what enables them to resist or bounce back from severe environmental stress. They've found tantalizing hints that heat-resistance genes, the proximity of other reefs, and even the presence of plant-eating sea life that scrub corals free of weedy algae can play a role. But they're also discovering that the factors that promote resilience can vary greatly from reef to reef.

Still, coral researchers are trying to extract some general, widely applicable lessons from their studies of resilience, partly in hopes of developing smarter conservation strategies,

such as better designed marine reserves. It's a task that is taking on increased urgency, as climate change threatens to wipe out corals and remake ocean ecosystems. "We in the environmental community look for points of hope," says ecologist Stephanie Wear of the Nature Conservancy in Arlington, Virginia. And in identifying possible ways to boost reef resilience, she says, "We see opportunity."

Stressful developments

Coral reefs weren't always considered fragile. When Rogers was working on her doctorate in the late 1970s, many researchers believed that reefs were intrinsically stable ecosystems, threatened mainly by local storm damage. But by the early 1980s, it was obvious that corals were in trouble. In the Caribbean, reefs took a noticeable hit after disease led to a massive die-off of spiny sea urchins, which had helped to keep reef-smothering algae in check after another group of herbivores, parrotfish, had been overexploited. In the Pacific and Indian oceans, unusually high seawater temperatures linked to the large-scale weather pattern known as El Niño caused massive "bleaching" events in 1982 and 1998 that turned reefs a ghostly, skeletal white. Bleaching happens when heat-stressed corals expel the symbiotic algae—

known as zooxanthellae—which live in their tissues and supply nutrients in exchange for protection. And it is often fatal: the 1998 event killed 16% of the world's reefs overall and up to 95% in some locations, says Robert Steneck, a biologist at the University of Maine's Darling Marine Center in Walpole.

But here and there, reefs have shown the ability to resist or rebound from such shocks. In an oft-cited example, the extensive reef system off Palau, in the western Pacific, charged back within a decade after suffering extensive bleaching losses in 1998. Similarly, reefs off the Cocos Islands in the eastern Pacific, which were virtually destroyed by bleaching during the 1980s, experienced up to fivefold increases in coral cover within 20 years. These recoveries sparked widespread interest in understanding the underlying "drivers" of resilience, with an eye toward developing better reef protection plans.

Scraping by

One focus has been on understanding the role of herbivorous fish and plant-eating invertebrates such as sea urchins. Ecologists have long known that these grazers play an important role in reef health by mowing down weedy algae and clearing attractive settling spots for young corals. Now, many believe that those tasks are essential to enabling some damaged reefs to recover from ecological stress.

For example, when Steneck visited Palau's bleached reefs in 2000, he was heartened by the abundance and diversity of herbivorous fish that were still patrolling the coral skeletons. "The reefs were extremely well-grazed," he recalls. "I thought the conditions for coral recovery were great because young corals would have a chance to settle." The subsequent recovery of those reefs reinforced Steneck's belief that protecting herbivorous fish is one of the most effective means of boosting reef resilience. A number of studies appear to back his view, including experiments published in *Current Biology* in 2007 conducted on Australia's Great Barrier Reef in which researchers deliberately removed grazing fish; the corals were soon overwhelmed by algae.

At the same time, in other parts of the

world, the importance of grazer abundance is less obvious. Researchers have found no clear relationship, for instance, between the number of reef herbivores and how much macroalgae covers reefs off the coast of New Caledonia, in the Southwest Pacific, a team led by Laure Carassou, a postdoctoral research fellow at Rhodes University in South Africa, reports in a forthcoming *PLOS*



Different fates. Corals in Palau (top) have recovered from severe bleaching, while many reefs in the Caribbean (bottom) have not.

ONE paper. Even on reefs where herbivorous fish numbers had been severely depressed by overfishing—such as in the Coral Triangle, the vast marine system that stretches from the Southwest Pacific to the western tip of Indonesia—algae cover tends to be low, notes co-author Michel Kulbicki, a reef ecologist with the Research Institute for Development in Banyuls, France. Such results suggest something is still eating the algae, and that conservation plans may need to target those species—rather than all herbivores—to boost resilience.

Making connections

Researchers are finding similar complexities as they explore the role of “connectivity,” or how ocean currents and oceanographic corri-

dors enable organisms, especially juvenile fish and corals, to move from one reef to another. In general, connectivity has been seen as crucial to maintaining reef resilience, because it can enable a damaged reef to receive a steady supply of fresh recruits from even distant reefs that are still healthy. That’s why conservation planners often seek to design marine reserves so that they protect both the nursery sources and the final homes of reef organisms. The problem with that approach, studies suggest, is that some damaged reefs may not be able to count on immigrants to make up for coral losses. Many coral larvae appear to travel no more than 300 meters before settling down, notes Maine’s Steneck, and there is growing evidence that many reefs “self-seed,” or produce their own recruits. That reproduction strategy presents a challenge to coral conservationists, because self-seeding reefs are thought to be less resilient than more connected reefs. For example, isolated reefs in the eastern Pacific—which rely on self-seeding by geographic necessity—recover more slowly from disturbances than better connected reefs to the western Pacific, according to biologist Nicholas Graham of James Cook University, Townsville, in Australia.

Hot topic

To get at the biochemical foundations of resilience, researchers have also been delving into coral physiology and genetics, particularly to understand how some reefs have avoided bleaching. In a sheltered reef pool off the coast of an island in Samoa, for instance, researchers in 2008 discovered corals that thrive in unusually warm water that regularly reaches 37°C, some 7° above the warmest summer temperature of nearby seas. That’s remarkable because corals typically bleach at just one degree above the local summer maximum, notes marine biologist Stephen Palumbi of Stanford University in Palo Alto, California.

As part of their study of these atypical corals, Palumbi’s team examined which genes switched on when confronted with heat stress. In all, the tests identified 61 stress-resistance genes, including those that code for heat-shock proteins, antioxidant enzymes, and immune regulators, the team reported in January in the *Proceedings of the National Academy of Sciences*. The next step, researchers say, is to see how many other corals might carry similar genes that could confer resilience.

Some good candidates might come from places where seawater temperatures already vary greatly, providing a kind of evolutionary “tough love” that could prepare corals to

deal with warming seas, says zoologist Tim McClanahan of the New York City–based Wildlife Conservation Society. Off the west coast of Madagascar, for instance, reefs are sheltered from a major cooling current that flows from the east, allowing them to thrive in waters that are often as warm as those encountered during El Niño events.

The hardy corals in St. John’s Hurricane Hole may have also benefited from heat-tolerance genes, since the water there is often warmer than it is around nearby offshore reefs, says USGS’s Rogers. But they also may have been helped by shading from nearby mangrove trees, she says, given that exposure to ultraviolet light is known to exacerbate the effects of warmer waters on coral bleaching. (Studies by ecologist Peter Mumby of the University of Queensland in Australia suggest that coral reefs surrounding the Society Islands, in the western Pacific, escaped the 1998 bleaching event because they were protected by cloud cover.)

Lessons learned?

In a bid to draw some broad, practical lessons from these growing but scattered examples of resilience, McClanahan recently asked 50 colleagues to help him develop a list of the top factors that might predict which reefs can resist and recover from bleaching and other threats. He asked each scientist to rank 31 resilience drivers and give the top scores to those they felt had the most scientific evidence. The result, published online last August in *PLOS ONE*, was a list of 11 highly ranked drivers. The researchers said a reef could have better recovery prospects, for instance, if it had a high level of coral recruitment and a low level of macroalgae. And reefs might be able to resist bleaching if they were already home to varieties of zooxanthellae or corals known to tolerate warmer waters; corals from the genus *Porites*, for instance, don’t bleach as readily as other species.

McClanahan’s study is prompting some government agencies that are responsible for managing corals—such as the U.S. National Oceanic and Atmospheric Administration (NOAA)—to try to characterize reefs by their resilience potential. The idea is to ultimately come up with site-specific protection plans that take into account a reef’s strengths and seek to shore up weaknesses. For example, research suggests that sediment and nutrient pollution can diminish a coral’s tolerance to heat, elevating bleaching risks. By addressing those land-based threats, managers could shore up a reef’s resilience to warming seas, explains

CREDITS (TOP TO BOTTOM): ROBERT STENECK/UNIVERSITY OF MAINE; CAROLINE ROGERS/USGS, ST. JOHN, U.S. VIRGIN ISLANDS

NOAA marine scientist Britt Parker in Silver Spring, Maryland.

There are numerous hurdles, however, to putting resilience-based plans into practice. One is that managers often lack key pieces of knowledge, such as exactly where young corals and fish come from on a given reef. So if they are trying to design a new preserve, that means they may not know which other reefs to include to ensure adequate connectivity. To get around such problems, conservation planners sometimes use proxies—such as data on currents or the knowledge of local people about fish populations—to help identify the right areas to protect. One risk in that approach, however, is that it can lead to proposed reserves that are so big that they stoke opposition from anglers, divers, and others who might lose access. Such conflicts might be reduced, Wear says, if researchers were able to develop inexpensive, noninvasive ways to establish reef connectivity (such as easy genetic tests) and fine-tune reserve designs.

A better understanding of resilience could help planners develop more targeted conservation strategies that don't threaten local livelihoods, Steneck agrees. For example, if studies show that the presence of herbivorous fish are essential to a reef's health, but carnivorous fish play a lesser role, officials could consider customized fishing limits. The Caribbean island of Bonaire, for instance, outlaws spearfishing and trap fishing, which can target herbivorous reef fish, but allows bait-and-hook fishing for carnivores. And in Palau, officials have banned the export of herbivorous parrotfish, but allow killing of the fish for local consumption. "The reef off Palau is huge, but the local [human] population is small, so there are plenty of parrotfish to graze the reef and sustain local needs," Steneck explains. "But no reef population could withstand fishing pressure for export markets."

Long-term challenges

Even if managers are successful in injecting resilience-based measures into conservation plans, however, it is not clear that they can protect reefs from the twin long-term challenges posed by rapid climate change: rising water temperatures and ocean acidifi-



Gone for good? Researchers are trying to understand what allows bleached corals, such as these off St. Croix in 2005, to recover.

cation, a pH change spurred by the sea's absorption of atmospheric carbon dioxide. The discovery of heat-tolerant corals is giving some researchers hope that some species will adapt to warmer seas. In addition to the Heat-resistant Palau corals, researchers have identified reef-building corals at the northern end of Australia's Great Barrier Reef that tolerate warmer water temperatures than

move by 15 kilometers per year, with their complicated ecosystems intact, to stay within the temperatures to which they're acclimated, he reported in an August 2012 study published in *Nature Climate Change*.

Another sobering unknown is whether corals can keep pace with acidifying seas, which can dissolve the calcium skeletons of corals and interfere with the development of eggs and larvae. Coral calcification will no longer keep pace with physical reef erosion once atmospheric carbon dioxide levels top 450 parts per million, according to Hoegh-Guldberg. With

levels now at 395 ppm, and rising at a rate of 2 ppm a year, that tipping point could be less than 30 years away, he notes.

Researchers have found hints that some corals and reef organisms have genetic resilience to acidification. Both Palumbi and Hoegh-Guldberg say that they have collected data showing that corals respond to acidification by changing gene expression, as they do when exposed to warmer seas. "But identifying a genetic response is a long way from showing that a coral can adapt—and that coral reefs can survive—the extremely rapid pace at which we are changing the environment," Hoegh-Guldberg says.

With conditions changing so quickly, Hoegh-Guldberg and other scientist have suggested that the world's reefs might be saved only with more radical measures to enhance resilience: by breeding heat-resistant corals and using them to build new reefs, for instance dumping minerals into the sea to neutralize acidity; or even shading reefs with vast sheets of buoyant cloth. But such measures are probably impractical, given the vast extent of reefs.

In the meantime, resilience studies are reinforcing the need for multifaceted conservation strategies that look for both short- and long-term gains. "In some ways, what we are learning about coral resilience is simply bringing us back to where we started," USGS's Rogers says. "Managing human activities at the local level—while still hoping that global efforts to control greenhouse gas emissions will become more effective."

—CHARLES SCHMIDT

Charles Schmidt is a writer living in Portland, Maine.

Keys to Reef Resilience

In general, researchers say that reefs with high fish and coral diversity and low human impacts are most likely to resist or recover from environmental stress. Other factors include:

Resistance

- Presence of heat-tolerant coral and symbiont species
- High water temperature variability, which can promote heat tolerance

Recovery

- High levels of coral recruitment to replenish denuded locations
- Suitable substrate for coral settlement and survival

those found 1500 miles to the south. But to survive, reefs may also have to migrate to cooler waters—and that's a doubtful proposition, says Ove Hoegh-Guldberg, director of the Global Change Institute at the University of Queensland. Reefs would have to



LETTERS

edited by Jennifer Sills

Misuse of Scientific Data in Wolf Policy

THE RECENT LAUNCH OF AN INTERNATIONAL PLATFORM FOR BIODIVERSITY SCIENTISTS AND policy-makers (IPBES) has raised hopes that a deeper consideration of scientific knowledge will lead to more efficient biodiversity conservation policies (1). However, this link is not systematic; the case of wolf conservation in Sweden reinforces concerns that scientific data can be misapplied to real-world problems.

Wolves in Sweden have been naturally recovering from near-extinction for the past three decades. All 250 wolves in Sweden descend from only 5 founders, and reducing the inbreeding coefficient has become the main policy target (2). However, the wolf recovery is controversial, and vocal interest groups have been calling to reduce population size because of negative impacts on hunting and farming.

Under pressure from these groups, Swedish authorities recently opened a wolf hunt, which selectively targets the most inbred wolves. This hunt is presented as a conservation action under the reasoning that removing some of the most inbred wolves is “the only measure in the short term that can reduce inbreeding” (2) and thus a step toward the species being able to maintain itself on a long-term basis, known as “Favorable Conservation Status” under binding EU legislation.

Unfortunately, this is just one example of how scientific results can be misinterpreted to justify a particular policy by ignoring the broader scientific context. In fact, only immigration will lead to a lasting reduction of inbreeding (3), but plans to support immigration have either failed or remain very uncertain.

If found lawful by a pending court case, this approach may have far-reaching consequences for biodiversity conservation in Europe, as it may legitimize the government's selective use of biodiversity science to spuriously justify biodiversity-damaging policies. To ensure that science is considered in context by policy-makers, scientists must provide more targeted warnings against the misuse of their results.

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Biodiversity Depends on
Logging Recovery Time

IN THEIR LETTER “BIODIVERSITY DESPITE selective logging” (8 February, p. 646), D. P. Edwards and W. F. Laurance extol the virtues of selectively logged forests in capturing tropical biodiversity, but point out that they may be more vulnerable to clear-cuts or fires compared with unlogged primary forests. Their arguments are very pertinent in understanding the conservation importance of logging-disturbed forests, especially given the growing number of timber concessions in neotropical forests. However, we feel that by overlooking the interactions between logging and other anthropogenic perturbations, the authors have presented an overly simplified view.

The world's total forest area is just over 4 billion hectares, 30% of which is managed primarily for timber and nontimber production (1). It is true that selective logging is a lesser evil that results in less forest damage and higher biodiversity retention (2) than many alternative land uses. However, this assumes that once-logged forests can recover to approximate preharvest baselines of forest biomass and species composition, if they can be left largely undisturbed. Sadly, this recovery process is rarely allowed to run its course. Many postlogging forest areas inevitably enter an irreversible sequence of forest degradation events, as they are typically more likely to be subjected to a new cutting cycle (3), wildfires underneath the forest canopy (4), trees naturally uprooted by wind (5), and new settlements by colonists and land speculators (6).

The Brazilian Amazon holds the largest remaining stock of tropical timber and is the world's richest treasure trove of terrestrial biodiversity (7). However, the best approach to ensure the long-term persistence of Amazonian biodiversity remains intensely debated. Although the number and extent of Amazonian protected areas have increased in the past decades, most of these are sustainable-use

Letters to the Editor

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reserves where timber and nontimber extraction is often poorly regulated (8). Meanwhile, since 2006, the Brazilian government has continued with a development program that allocates tracts of public lands as large-scale forest management concessions. Some 1.3 million hectares of forests have already been allocated to timber concessions in National Forests alone (9). This program may suppress illegal logging, but so far has been primarily concerned with the management protocol of the first logging cycle, with little or no concern for the prolonged postlogging recovery trajectory.

Without a proper long-term forest management program that explicitly considers both the timber extraction and postlogging phases, the long-term coexistence of logged forests and their high biodiversity value will remain highly questionable.

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TECHNICAL COMMENT ABSTRACTS

Comment on "Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage"

Shigeki Nakagome, Shuhei Mano, Masami Hasegawa

Based on nuclear and mitochondrial DNA, Hailer *et al.* (Reports, 20 April 2012, p. 344) suggested early divergence of polar bears from a common ancestor with brown bears and subsequent introgression. Our population genetic analysis that traces each of the genealogies in the independent nuclear loci does not support the evolutionary model proposed by the authors.

Full text at <http://dx.doi.org/10.1126/science.1227339>

Response to Comment on "Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage"

Frank Hailer, Verena E. Kutschera, Björn M. Hallström, Steven R. Fain, Jennifer A. Leonard, Ulfur Arnason, Axel Janke

Nakagome *et al.* reanalyzed some of our data and assert that we cannot refute the mitochondrial DNA-based scenario for polar bear evolution. Their single-locus test statistic is strongly affected by introgression and incomplete lineage sorting, whereas our multilocus approaches are better suited to recover the true species relationships. Indeed, our sister-lineage model receives high support in a Bayesian model comparison.

Full text at <http://dx.doi.org/10.1126/science.1228066>

CORRECTIONS AND CLARIFICATIONS

Review: "Neutralizing tumor-promoting chronic inflammation: A magic bullet?" by L. M. Coussens *et al.* (18 January, p. 286). Reference 52 cites the wrong paper. Instead, the reference should be J. Coward *et al.*, *Clin. Can. Res.* **17**, 6083 (2011). The HTML and PDF versions online have been corrected.

Reports: "Evolution of an MCM complex in flies that promotes meiotic crossovers by blocking BLM helicase" by K. P. Kohl *et al.* (7 December 2012, p. 1363). In Fig. 2A, the scale in the vertical axis should have appeared as 0 to 25, not 0 to 50. The bars remain in the same positions relative to each other, and the conclusions of the Report are unaffected.



theBUZZ

Genetically Modified Organism Policy

In the 22 February Editorial, "The GMO stalemate in Europe" (p. 883), L. O. Fresco bemoans the European Union's (EU's) reluctance to embrace genetic modification in agriculture. The article received many comments from those who feel the EU's caution is well placed, and a few who disagree. An example of each opinion is below. Read the full comments at <http://comments.sciencemag.org/content/10.1126/science.1236010>.

A selection of your thoughts:

Ultimately what one eats determines the state of one's health. The Europeans seem to care about what they eat, and they are wise to adopt the precautionary principle. GM foods should not be consumed by any organism until they can be shown beyond reasonable doubt that they are safe in the short and long run. More research funds should be channeled to encourage scientists to study the effects of GMOs on organisms as well as the environment, independently and without corporate influence.

—Shu-K. Yang

...The critics of GM technology link its use to other questions (globalization, agrobusiness) rather than addressing the technology on its own merits.... We should emphasize the science behind the GM technology and how much GM is rooted in what organisms do naturally....

—Alan Schulman

Meanwhile, a Letter in the 15 February issue objected to AAAS's policy statement against mandating GM labeling. S. H. Priest *et al.* wrote that even if genetically modified organisms are shown to be harmless, people deserve access to the information, and withholding it could threaten the public's trust in science ("AAAS position on GM foods could backfire," p. 756). Commenters spoke up, some supporting AAAS's policy, and others agreeing with Priest *et al.* A comment representing each position follows. Read the full comments at <http://comments.sciencemag.org/content/10.1126/science.339.6121.756-a>.

A selection of your thoughts:

The AAAS took the correct position. Government-mandated food labels should contain information related to human health (nutrition) and safety. There is much information of this type—levels of mycotoxins, heavy metals such as lead, metalloids such as arsenic, and various allergens—that is already impossible to cram into the limited area of a single label. Transmitting information of this type to consumers can reduce food-related illness, which afflicts approximately one in six Americans every year. In contrast, there is no reputable data—none—indicating any greater health risk from crops grown with modern breeding methods vs. older methods. Label information with real nutrition and safety consequences should not make way for other forms of literature. Food packagers and retailers are free to add information, either on the package or in point-of-sale displays, that addresses the idiosyncratic, non-health-related requirements of their customers, whether it is information on the methods of crop breeding involved or the astrological sign of the crop harvest date.

—Bruce Ferguson

It is very disappointing that Science has gone with the big biochem/agribusiness companies that wield such power over researchers, media, and government. Their research is sloppy and secretive, and the long-term results unknown. To assume that massive use of glyphosphate and Roundup are harmless to the natural environment or the human body, as well as to small farmers and their communities, takes a gigantic leap of faith no prudent scientist would make.

—Elizabeth Sanders

Comment on “Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage”

Shigeki Nakagome,^{1*} Shuhei Mano,¹ Masami Hasegawa^{1,2}

Based on nuclear and mitochondrial DNA, Hailer *et al.* (Reports, 20 April 2012, p. 344) suggested early divergence of polar bears from a common ancestor with brown bears and subsequent introgression. Our population genetic analysis that traces each of the genealogies in the independent nuclear loci does not support the evolutionary model proposed by the authors.

Hailer *et al.* (1) sequenced 14 independent nuclear loci across the genomes of polar bears (*Ursus maritimus*), brown (*U. arctos*), and American black bears (*U. americanus*). The Bayesian multilocus coalescent approach showed one species tree in which polar bears split from

brown bears before the diversification of brown bear lineages. The divergence of polar bears was estimated to have occurred 603 thousand years ago (ka). These results conflict with the mitochondrial DNA phylogeny, which defines the root of the polar bear lineage within brown bear diversity (2, 3) (“standard model” in Fig. 1A). Additional phylogenetic analysis using the concatenated sequences supported the sister lineage of polar bears to all brown bears. Based on these observations, the authors proposed a new evolu-

tionary model in which polar bears diverged from the common ancestor of all extant brown bears, whereas hybridization with female brown bears facilitated introgression (“new model” in Fig. 1A).

Here, we focused on a unique genealogical history in each locus and applied population genetic analysis to the same data set evaluated in Hailer *et al.* (1), including 14 unlinked nuclear loci from brown bears ($2N = 36$) and polar bears ($2N = 36$). A crucial difference between the standard model and the new model is the time to the most recent common ancestor (MRCA) in brown bears ($T_{\text{MRCA-uar}}$) (Fig. 1A). In the standard model, the $T_{\text{MRCA-uar}}$ is the same for all of the samples from brown and polar bears ($T_{\text{MRCA-all}}$) ($T_{\text{MRCA-all}} = T_{\text{MRCA-uar}}$). The ratio of $T_{\text{MRCA-uar}}$ to $T_{\text{MRCA-all}}$ ($T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$) is expected to be 1.00 in this model. In contrast, the new model shows that the $T_{\text{MRCA-all}}$ is older than the $T_{\text{MRCA-uar}}$. From the estimates of $T_{\text{MRCA-all}}$ (603 ka) and $T_{\text{MRCA-uar}}$ (125 ka), Hailer *et al.* (1) estimated the ratio of 0.21. We used the $T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$ as a test statistic to ask whether the observed genetic variation significantly deviates from the null hypothesis of the standard model.

We estimated the $T_{\text{MRCA-all}}$, $T_{\text{MRCA-uar}}$, and T_{MRCA} in polar bears ($T_{\text{MRCA-uma}}$) for 13 loci using the GENETREE program (4) (Table 1).

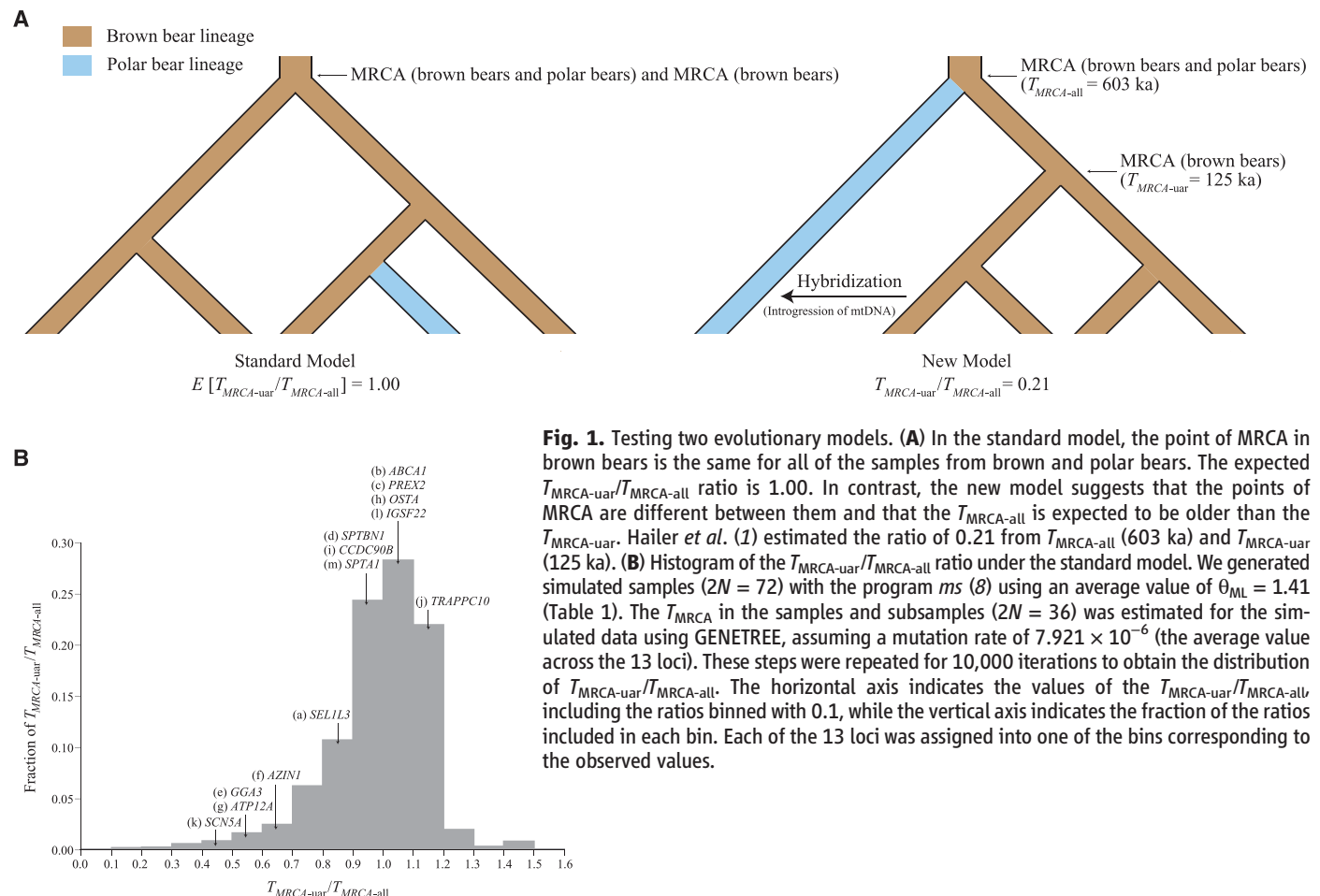


Fig. 1. Testing two evolutionary models. **(A)** In the standard model, the point of MRCA in brown bears is the same for all of the samples from brown and polar bears. The expected $T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$ ratio is 1.00. In contrast, the new model suggests that the points of MRCA are different between them and that the $T_{\text{MRCA-all}}$ is expected to be older than the $T_{\text{MRCA-uar}}$. Hailer *et al.* (1) estimated the ratio of 0.21 from $T_{\text{MRCA-all}}$ (603 ka) and $T_{\text{MRCA-uar}}$ (125 ka). **(B)** Histogram of the $T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$ ratio under the standard model. We generated simulated samples ($2N = 72$) with the program *ms* (8) using an average value of $\theta_{\text{ML}} = 1.41$ (Table 1). The T_{MRCA} in the samples and subsamples ($2N = 36$) was estimated for the simulated data using GENETREE, assuming a mutation rate of 7.921×10^{-6} (the average value across the 13 loci). These steps were repeated for 10,000 iterations to obtain the distribution of $T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$. The horizontal axis indicates the values of the $T_{\text{MRCA-uar}}/T_{\text{MRCA-all}}$, including the ratios binned with 0.1, while the vertical axis indicates the fraction of the ratios included in each bin. Each of the 13 loci was assigned into one of the bins corresponding to the observed values.

Table 1. GENETREE analysis for brown and polar bears, brown bears, and polar bears. Insertion and deletion polymorphisms were excluded in the GENETREE analysis. Recombinant sequences were also excluded in this analysis based on the infinite-site model. We confirmed that most of the recombinants were rare in a population ($n = 1, 2$, or 3) and that the segregating sites involved in the exclusion were also rare, which were

thought to be recent mutations. These data are unlikely to affect our estimation of the T_{MRCA} or alter the overall results. The *LRGUK* locus was not included in this analysis because there were many recombinants with high frequencies in brown bears. N.A. denotes that coalescent times could not be assessed due to the lack of a mutation. μ , mutation rate per locus per generation; N_e , effective population size.

Loci	μ^*	Brown and polar bears				Brown bears				Polar bears				Ratio of $T_{MRCA-uar} / T_{MRCA-all}$	$P^\dagger$ (threshold: $P = 0.004^\ddagger$)
		$S^\dagger$	$\theta_{ML}^\ddagger$	$N_e^\S$	$T_{MRCA-all}^\parallel$	S	θ_{ML}	N_e	$T_{MRCA-uar}$	S	θ_{ML}	N_e	$T_{MRCA-uma}$		
(a) <i>SEL1L3</i> **	6.869×10^{-6}	9	1.70	61,870	2,992,294	6	1.30	47,313	2,456,657	3	0.75	27,296	907,145	0.821	0.133
(b) <i>ABCA1</i>	6.366×10^{-6}	9	1.80	70,691	3,509,803	8	1.90	74,618	3,616,146	1	0.25	9,818	344,972	1.030	0.554
(c) <i>PREX2</i>	8.270×10^{-6}	8	1.30	39,300	2,631,307	8	1.60	48,369	2,818,974	0	N.A.	N.A.	N.A.	1.071	0.634
(d) <i>SPTBN1</i>	6.505×10^{-6}	10	2.10	80,712	2,566,795	7	1.70	65,338	2,486,114	2	0.50	19,217	695,582	0.969	0.369
(e) <i>GGA3</i>	6.921×10^{-6}	6	1.10	39,732	1,866,630	3	0.80	28,896	953,347	0	N.A.	N.A.	N.A.	0.511	0.018
(f) <i>AZIN1</i>	5.405×10^{-6}	5	1.10	50,878	1,760,479	3	0.80	37,002	1,090,971	1	0.22	10,176	486,190	0.620	0.036
(g) <i>ATP12A</i> ††	8.015×10^{-6}	8	1.80	56,144	1,784,831	4	1.10	34,310	1,040,019	2	0.50	15,596	538,955	0.583	0.029
(h) <i>OSTA</i>	8.623×10^{-6}	6	1.10	31,893	1,626,396	5	1.10	31,893	1,677,361	1	0.25	7,248	260,084	1.031	0.557
(i) <i>CCDC90B</i>	6.053×10^{-6}	5	1.00	41,300	1,563,955	4	1.00	41,300	1,502,253	3	0.54	22,302	1,475,775	0.961	0.355
(j) <i>TRAPPC10</i>	1.025×10^{-5}	7	1.30	31,711	1,407,140	7	1.20	29,272	1,686,337	0	N.A.	N.A.	N.A.	1.198	0.968
(k) <i>SCN5A</i>	8.623×10^{-6}	5	0.90	26,094	1,366,802	2	0.44	12,757	578,660	0	N.A.	N.A.	N.A.	0.423	0.011
(l) <i>IGSF22</i>	8.131×10^{-6}	5	1.00	30,747	1,274,600	5	1.30	39,972	1,369,025	0	N.A.	N.A.	N.A.	1.074	0.645
(m) <i>SPTA1</i>	1.295×10^{-5}	10	2.10	40,554	1,370,006	8	2.00	38,623	1,309,866	2	0.50	9,656	318,873	0.956	0.345
Average	7.921×10^{-6}	7	1.41	46,279	1,978,541	5	1.25	40,743	1,737,364	1	0.44	15,164	628,447	0.865	

*Mutation rates were calculated from the number of substitutions between the giant panda and brown and/or polar bears, assuming the divergence of the giant panda and brown/polar bears 12 Ma. †The number of segregating sites in a population. ‡Maximum-likelihood estimates of the scaled population mutation rate, θ . § N_e was calculated from $\theta_{ML} = 4N_e\mu$. || T_{MRCA} was calculated from $T_{MRCA} = 2N_e t \times 10$ (years/generation), where t is the time in coalescent-time units estimated by GENETREE. ¶Empirical P values represent the fraction of ratios ($T_{MRCA-uar}/T_{MRCA-all}$) less than the observed ratio at each locus to 10,000 simulated points. ††The significance threshold was corrected using the Bonferroni correction. **The total aligned sequences of 615 base pairs in the *SEL1L3* locus included recombinants from brown bears, and nucleotide positions from 1 to 395 were used for the GENETREE analysis. ‡‡One polymorphic site at the *ATP12A* locus was excluded due to an unknown state of an ancestral allele (the giant panda with "A" and the brown and polar bears with "G/C").

One locus, *LRGUK*, from Hailer *et al.* (1), was excluded in this analysis due to high frequencies of recombinants, whereas only recombinant sequences were excluded in the other loci. A genealogy of each locus was deduced based on the path of mutations to the MRCA under the infinitely many-site model. We computed the maximum likelihood estimates for the population mutation rate (θ_{ML}) under the constant size model in which we specified the range of θ_{ML} (5). The empirical distribution of the T_{MRCA} was obtained based on the likelihood estimated from each simulation run, conditional on the topology of the gene tree and θ_{ML} . The ancestral/derived state of an allele at a segregating site was inferred by alignment with the giant panda sequence (6). The genealogies were different for the 13 loci, and each locus had its own T_{MRCA} . Nine of the 13 loci showed that $T_{MRCA-uar}/T_{MRCA-all}$ ratios were 0.821 to 1.198. These results appear to support the standard model and the expected $T_{MRCA-uar}/T_{MRCA-all}$ value of 1.00.

To determine whether the observed values of the $T_{MRCA-uar}/T_{MRCA-all}$ could reject the null hypothesis, we generated a distribution of the ratios under the standard model (Fig. 1B). Empirical P values were calculated for the 13 loci (Table 1). The estimate in Hailer *et al.* (1) ($T_{MRCA-uar}/T_{MRCA-all} =$

0.21) significantly deviates from the null distribution ($P = 0.002$). However, we found that all of the P values for the 13 loci were greater than the threshold for significance (corrected $P = 0.004$) and that the standard model could not be rejected. The estimates for the $T_{MRCA-uma}$ were consistently lower than the estimates for the $T_{MRCA-all}$ and $T_{MRCA-uar}$, which is consistent with the standard model because the $T_{MRCA-uma}$ is expected to be more recent than the $T_{MRCA-all}$ and $T_{MRCA-uar}$. These results provide more information on relevant arguments against the new model.

A recent study using a diploid genome pointed out that hundreds of thousands of independent loci within an individual have different T_{MRCA} (7). Our estimations of the $T_{MRCA-all}$ are 1.3 to 3.5 million years ago (Ma), whereas the $T_{MRCA-uma}$ is estimated to be 0.3 to 1.5 Ma (Table 1). Genealogies with $T_{MRCA-all}$ older than 1.5 Ma may be useful for tracing the population history before the divergence of brown and polar bears. The genealogies of the *SEL1L3*, *ABCA1*, *PREX2*, and *SPTBN1* loci indicate that the lineages leading to polar bears occurred during the diversification of brown bear lineages, which supports the standard model. Our population genetic analysis indicates that the observed patterns of genetic variation in nuclear loci can be explained

by the recent origin of polar bears and ancestral polymorphisms.

In summary, we conclude that the 13 loci reported by Hailer *et al.* (1) fail to support the new model. The sequence data from the 13 loci are not sufficient to resolve the origin of the bears. Genome-scale sequence data are necessary to untangle the complex evolutionary history of brown bears and polar bears.

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Frank Hailer,^{1*} Verena E. Kutschera,¹ Björn M. Hallström,^{1,2} Steven R. Fain,³ Jennifer A. Leonard,⁴ Ulfur Arnason,⁵ Axel Janke^{1,6*}

Nakagome *et al.* reanalyzed some of our data and assert that we cannot refute the mitochondrial DNA-based scenario for polar bear evolution. Their single-locus test statistic is strongly affected by introgression and incomplete lineage sorting, whereas our multilocus approaches are better suited to recover the true species relationships. Indeed, our sister-lineage model receives high support in a Bayesian model comparison.

Studies of mitochondrial DNA (mtDNA) in polar and brown bears show polar bears deeply nested within brown bear diversity [paraphyly; “standard model” in (1)], with an origin of the extant polar bear lineage around 111 to 166 thousand years ago (ka) (2, 3). Recently, we showed that polar bears form a distinct sister lineage to brown bears based on genomic variation at 14 independently inherited nuclear loci (4) [Fig. 1A; “new model” in (1)] and dated their speciation to 338 to 934 ka using multilocus methods.

Nakagome *et al.* (1) argue that our results cannot reject the standard model based on re-

analysis of a subset of our data (see Fig. 1B) for two reasons:

(i) Time to the most recent common ancestor (T_{MRCA}) of polar bears is smaller than T_{MRCA} of brown bears and of brown and polar bears combined.

This observation is entirely compatible with both the standard model and the new model. T_{MRCA} is proportional to effective population size (N_e). Polar bears have a much smaller population size than brown bears and thus have a shorter expected time to coalescence. Intraspecific T_{MRCA} values therefore reflect population size rather than divergence time from another bear species.

(ii) The T_{MRCA} ratios (1) for individual loci do not conform to the multilocus expectations based on the new model (T_{MRCA} ratio = 0.21).

This approach implicitly assumes that if a species tree shows reciprocal monophyly among taxa (Fig. 1A), then individual gene trees will show that same topology. However, gene trees and species trees are not always the same, and coalescence theory predicts that it takes around $4 N_e$ generations to complete lineage sorting at nuclear loci (5). In bears, this corresponds to 1.12 million years, assuming an effective pop-

ulation size of 28,000 (1, 6) and a generation time of 10 years (7, 8). Lineage sorting is thus expected to be incomplete among polar and brown bears, leaving many of their alleles intermingled at gene trees of individual loci [incomplete lineage sorting (ILS)] (Fig. 1B). Similarly, a genomic comparison of human, chimp, and gorilla demonstrated that 30% of bases in their genomes exhibit ILS (9) on time scales of several million years.

Introgression is also known to affect lineage sorting and, thus, T_{MRCA} of DNA sequences (Fig. 1C). Hybridization between polar and brown bears has been confirmed in the wild (10), and clustering of polar bear mtDNA within brown bear diversity likely reflects introgressive mitochondrial replacement in polar bears (3, 4, 6). Gene flow signals from polar into brown bears were present in our data (Fig. 2B), and (6) found 5 to 10% of the genome of some brown bears to be introgressed from polar bears. Hence, due to ILS and introgression, many nuclear loci are not expected to show reciprocal monophyly under either scenario of polar bear evolution. Thus, T_{MRCA} patterns for many individual loci will deviate from the species tree, and $T_{MRCA-uar}$ will approach $T_{MRCA-all}$ (Fig. 1C), as observed in (1). This likely explains why T_{MRCA} ratios of many loci are close to 1 (1), without contradicting the new model.

The analysis of Nakagome *et al.* did not include the black bear sequences that were part of our original paper (4). Black bears diverged from brown and polar bears approximately 1 to 5 million years ago (4, 6). Nevertheless, five out of 41 (12%) black bear alleles at nuclear introns were shared with brown/polar bears (4), and in many cases they did not cluster ancestrally (Fig. 2). Inclusion of black bear data in the analyses is important, because the results highlight the impact of ILS and introgression in bears on time scales far beyond a few 100 ka (Fig. 1B).

Phylogenetics research has moved toward interpreting gene trees as local optima that show snapshots of the evolutionary history of the studied taxa (11). Recognizing that gene trees do not always reflect species trees, inference of

Fig. 1. Hypothetical gene trees in bears. (A) Idealized phylogeny of black, brown, and polar bear genes; the topology is identical to our species tree (4). This topology does not show introgression and ILS at the single-gene level. (B) Inclusion of black bear sequences (stippled lines) highlights the impact of ILS/introgression on gene trees in bears. Without black bears (1), the gene tree appears to have the standard model topology—i.e., polar bears nested within brown bears (see Fig. 2C for real data showing an analogous pattern). (C) Impact of introgression (stippled line) from polar into brown bears. Introgression equalizes the T_{MRCA} values of (i) brown bears and (ii) brown and polar bears combined.

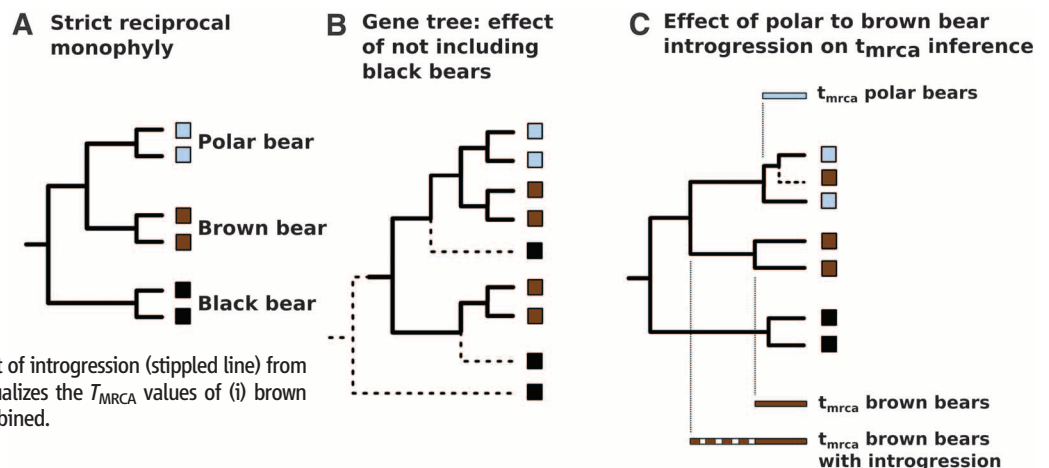
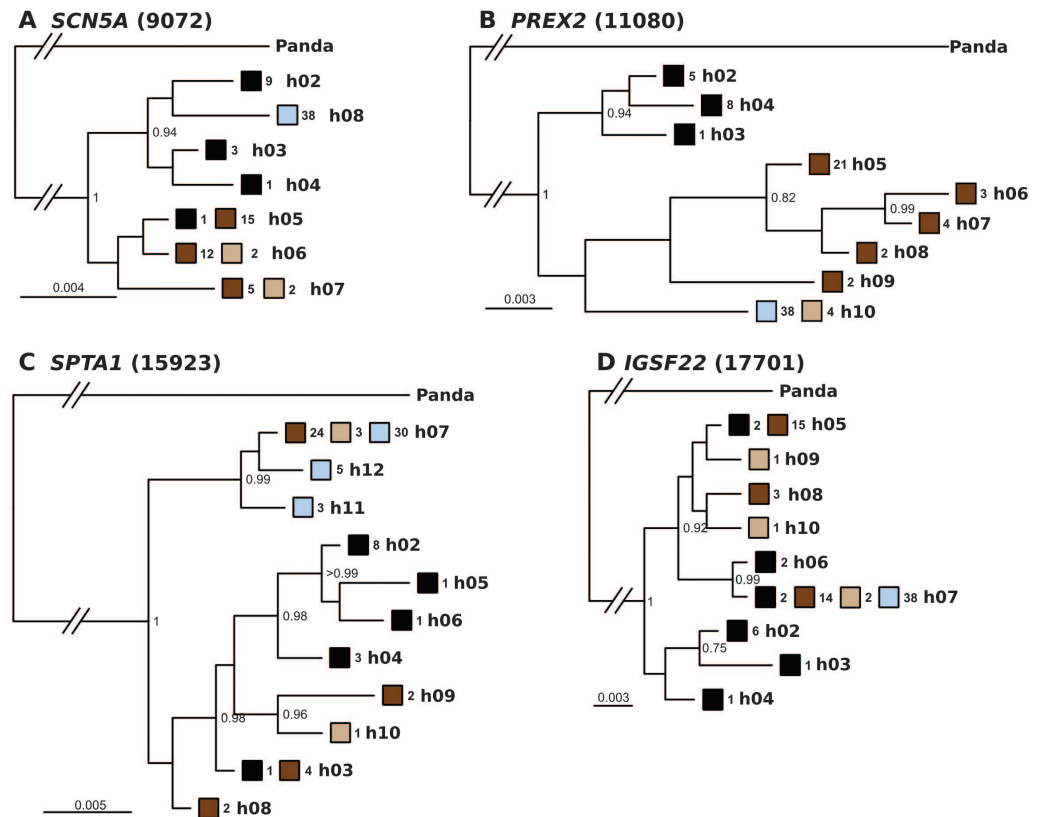


Fig. 2. Representative gene trees in bears, based on our data (4). (A to D) In each case except (B) (evidence of polar to brown bear introgression), inclusion of the black bear sequences is crucial for correct interpretation of clades, highlighting the impact of ILS and/or introgression on the gene trees. Gene names and corresponding locus identifications from (4) are shown above each tree. The trees and support values (only values above 0.75 are shown) were obtained from Bayesian analyses (15) (4 heated chains, 200×10^6 generations each). Black, brown, and blue denote black, brown, and polar bear alleles, respectively. Light brown shows alleles in brown bears from the Alaskan ABC islands.



species trees as opposed to single gene trees has become an important goal (5, 11, 12). To this end, we jointly analyzed results from individual loci in multilocus analyses (4). Results from *BEAST (13), concatenation, and the ϕ_{ST} (genetic differentiation)-tree consistently recovered the same topology, with strong support for polar bears as a sister lineage to brown bears ($P > 0.99$), and black bears clustering ancestrally (4)—the new model. Inferences in *BEAST can also be affected by introgression. This makes our multi-locus estimate of the speciation time conservative (14), increasing the contrast between our findings and the standard model. Further, our species-tree based T_{MRCA} of brown bears divided by T_{MRCA} of brown/polar bears combined deviates significantly from expectations for single loci under the standard model ($P = 0.002$) (1). This confirms that our nuclear data, analyzed in a multilocus framework, capture a different signal than that predicted by the mtDNA-based standard model.

To explicitly investigate whether our data fit better to the new model [figure 1A in (4)] or whether the standard model was better or equally consistent with our data [as suggested by (1)], we performed new analyses using *BEAST. We enforced constraints on the species tree based on (i) the standard model, constraining polar bears to

form a monophyletic group with brown bears from the Alaskan Admiralty, Baranof, and Chichagof (ABC) islands (2, 3); and (ii) the new model, constraining monophyly of ABC-island brown bears with North American mainland brown bears [as in our unconstrained species tree (4)]. The latter was done to confirm that constraining a certain species tree topology per se did not affect the analysis. This confirmation enabled us to perform statistical model comparisons. Consistent with our original interpretation of polar bears being a distinct sister lineage to brown bears, the standard model received significantly lower statistical support than the constrained (ii) and unconstrained (4) new models (support for new model: both Bayes factors = 375). Furthermore, recent analyses of genomic data (6) have also recovered a sister-lineage relationship among polar and brown bears and interpreted polar bear mtDNA as introgressed from brown bears.

Our data thus strongly support the sister-lineage model, highlighting that the overall evolutionary history of recently evolved taxa is best portrayed by multilocus approaches with appropriate outgroup data, due to the complicating processes of ILS and introgression. “Recently evolved” can span time scales ranging from several hundred thousand to millions of years in

bears, and even longer in species with larger effective population sizes.

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SOCIAL SCIENCES

The Price of Objectivity

Audra J. Wolfe

In the summer of 2011, the U.S. House of Representatives threatened to defund the National Science Foundation's Directorate for Social, Behavioral, and Economic Sciences (1). That effort failed, but the following spring the Republican-led House continued its assault with a measure barring the NSF from funding research in political science. Conservative critics, led by Representative Jeff Flake (R-AZ), charged that too much of the NSF's budget was supporting politically biased research that offered little social benefit beyond satisfying the "curiosities of a few academics" (2).

Such charges have dogged the NSF's programs in the social sciences from the agency's earliest days. Nor has the NSF been the only target: during the first decade of the Cold War, congressional critics charged that both the Pentagon and private philanthropic foundations were sponsoring work in the social sciences that either served no useful purpose or undermined American values. In *Shaky Foundations*, Mark Solovey charts how these ongoing attacks forged the claims to objectivity so characteristic of the postwar social sciences. As federal and philanthropic support for basic research in psychology, sociology, anthropology, and economics grew in the 1950s and 1960s, entrepreneurial social scientific researchers insisted that their newly "rigorous, systematic, and quantitative" methods could offer "apolitical, nonideological, and value free" tools to improve the nation's welfare and strengthen its defense. Having supposedly mastered data collection and analysis techniques developed by the natural sciences, the social sciences were well on their way to embracing what Solovey (a historian at the University of Toronto) refers to as a scientific worldview.

During the few short years that social scientists and their patrons at the Army, the Ford Foundation, and the NSF agreed on the utility of this approach, the American social sciences came the closest that they ever have to a blank check for fundamental research in human behavior—provided that research steered clear of such "social issues" as race, religion, or class. That is, of course, a pretty

big "but" for the so-called human sciences. Since the early 1960s, when New Left critics led by sociologist Irving Horowitz called out a militaristic bias in the work of economists and political scientists bankrolled by defense agencies, historians and social critics alike have uncovered the hidden costs of all this easy money. Solovey's account extends this tradition by "following the money," but his refreshing attention to his subjects' ambivalence adds a new layer to historians' understanding of the social sciences during the Cold War.

Solovey's social scientists are neither naïve researchers exploited by the military-industrial complex nor greedy masqueraders eagerly anticipating their patrons' needs. Instead, he presents us with a series of encounters between program managers, disciplinary spokesmen, and political partisans, each of which demonstrates its participants' unexpectedly complex positions. At each of

these moments—the founding of the NSF, the military's grudging acceptance of psychologists on its Human Resources Committee, the Ford Foundation's decision to create (and later end) a Behavioral Sciences Program, and the NSF's halting embrace of "hard-core" social sciences in the late 1950s—the reader encounters subjects with startling levels of self-awareness. Solovey balances every H. Rowan Gaither, who suggested that the Ford

Foundation's projects in the behavioral sciences should be modeled after "a natural science like physics," with a Donald David, who warned that Ford neglected "human motives and the forces which govern human relations" at its peril.

In what feels like a prelude to contemporary partisan investigations of the social sciences, *Shaky*

Foundations recounts numerous instances of McCarthy-era attacks on social scientists as leftist agitators. Less familiar, however, are complaints from the right that the social sciences were too scientific. The 1954 Reece Committee hearings in the U.S. House, for instance, featured a series of witnesses who charged that social scientists' quest for value neutrality was leading the nation down a godless path. Conservative William F. Buckley Jr. similarly argued in 1952 that modern

sociologists and psychologists were trying to convert college students to "atheistic socialists." Critics on the left, meanwhile, charged that the commitment to "value-free" research had removed any impetus for social change. Against this background, the collapse of scientism in the 1960s seems not so much curious as inevitable.

Shaky Foundations ends there, with the mutual disenchantment of patron and client. As Solovey puts it, "assumptions about the apolitical and value-neutral character of legitimate scientific inquiry [seem] to be long gone and not likely to return any time soon." An unexplored irony of his account is the federal government's embrace of quantitative social scientific assessment measures at exactly the moment—the mid-1960s—that more theoretically inclined social scientists began to lose

Shaky Foundations

The Politics–Patronage–Social Science Nexus in Cold War America

by Mark Solovey

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Studies in Modern Science,
Technology, and the Environment.



Social-science weapon. A cluster adapter, packed with 22,500 psychological warfare leaflets, being prepared for dropping on North Korea (November 1950).

The reviewer is the author of *Competing with the Soviets: Science, Technology, and the State in Cold War America*. Web site: www.audrajwolfe.com

faith in objectivity. Republicans in Congress may not approve of NSF-funded research in political science, but they certainly endorse standards-based testing for education and cost-benefit analysis in environmental regulations. Those, too, are legacies of the Cold War social sciences.

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CONSERVATION BIOLOGY

A Wild Life

Harry W. Greene

Measured against our prevailing scientific culture—experiments, grants, high-impact journals, and tensions between teaching and research—George Schaller's career has been unconventional. At one level, *Tibet Wild* is a memoir of research on gorillas, lions, and other mammals in some of the most remote places on Earth. It spans eight decades, four continents, diverse species of large vertebrates, and accolades like the Tyler Prize for Environmental Achievement and the National Book Award for Science, yet none of it, beyond graduate school, takes place in a university setting.

On another level, this poignant autobiography circles around two interesting problems: What are the origins and consequences of such intensely personal commitment? And, more generally, how can we preserve biodiversity in the face of rapid global change?

Schaller, a self-described feral naturalist, was born in Berlin in 1933. As a boy, he scavenged food discarded by American soldiers, survived a harrowing escape through East Germany, and eventually settled in the United States. An introverted, difficult child who favored solitude, he was a bored, mediocre high school student until a summer exploring the Yukon set his course on the outdoors. Influential teachers also lit the way, first during undergraduate courses and fieldwork in

Alaska, then while he was a Ph.D. student at the University of Wisconsin. After dissertation work on gorillas, he landed a dream job with the Wildlife Conservation Society, for whom he conducted the first detailed studies of tigers, thence lions, giant pandas, and other species. The lion project, for example, entailed some 3000 hours of fieldwork, recalled as the happiest time of his life, to investigate trade-offs between solitary versus group hunting and the ecological impact of predators (1).

For more than three decades now, Schaller has worked in Asia, under some of the most demanding conditions ever endured by biologists. The Chang Tang is an enormous plain straddling Tibet's borders with the Xinjiang Uygur Autonomous Region and Qinghai Province, mostly uninhabited except for wildlife and small, widely scattered human settlements. Temperatures hover below -18°C and sometimes plunge to -34°C or worse, so even the simplest camp tasks can be hazardous due to exposure. Equipment regularly fails. Here, accompanied by Chinese colleagues on 26 trips over the course of 41 months, Schaller repeatedly has traversed distances the equivalent of New York City to Chicago. At one point, nearing the age of 70, he finally admitted, "Walking several hundred miles with a modest pack is no problem, but pulling a loaded cart at 17,000 feet through snow and mud, and up and down steep slopes is probably beyond me."

The Chang Tang is a Pleistocene Eden, home of the richest remaining megafauna outside of Africa—including wolf, brown bear, yak, and wild ass—of which an enigmatic antelope, the chiru, and a small rabbit relative, the plateau pika, most captured Schaller's attention. When he first arrived, all but the bare essentials of the chiru's natural history, even migration routes and birthing sites, were unknown. Its immediate future was threatened by hunting (during the 1990s, 250,000 to 300,000 were killed for their luxurious wool, made into expensive shahtoosh shawls), its ultimate survival imperiled by climate change. After all those brutal months and miles, Schaller could report that a "gathering of chiru on the calving ground ... was for me the most wondrous vision in a quarter century of following the species." On the same trips, he studied the highly social pikas and wrote a dozen charming, environmentally themed children's fables about them for translation into Tibetan.

Schaller emerged in *Stones of Silence* (2)



On the breeding grounds. Two male chiru (*Pantholops hodgsonii*) during their December mating season.

and other earlier accounts as decidedly taciturn, except when writing about his family or pandas and other noble animals. In *Tibet Wild*, he opens up on a wide range of personal matters, including Buddhist teachings as they relate to nature and existential questions of meaning. This latest volume begins with quotes from Basho, Laozi, and Ortega y Gasset, and throughout he writes affectionately of his wife and sons and of their home in an old New England barn, decorated with keepsakes from all over the world. A "cloud-walker," he "prefer[s] the beauty of a hermetic world suffused with stillness" and is content with "gaining new insights into a species, promoting the establishment of reserves, and stimulating young biologists to focus on conservation."

As for preserving biodiversity, Schaller's scientific efforts provide a historical baseline for vanishing species and ecosystems and his popular writings an example of how the aesthetic values of wilderness can enhance our lives. Moreover, although contemptuous of trophy hunting, he feels deep empathy for local people, including their consumption of and conflicts with the very animals he so obviously loves. Fail to address that complexity, he opines, and conservation is doomed. *Tibet Wild* offers a few such pronouncements, but mainly it lays out an open-ended account of the struggle to save wild places and their inhabitants. I can't recall any book that has made me care as much or think harder about how we might do that.

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Measuring China's Circular Economy

Yong Geng,^{1*} Joseph Sarkis,^{2,3} Sergio Ulgiati,⁴ Pan Zhang⁵

Unique environmental and economic challenges provide a laboratory for developing new indicator systems.

Facing significant natural resource consumption, environmental degradation, and resulting public frustration, China's new administration heightened attention on ecological modernization, green growth, and low carbon development, with a national circular economy (CE) strategy (1). The 2012 Rio+20 United Nations Conference on Sustainable Development emphasized the need to develop indicators of progress that decouple economic growth and environmental burden (2). We describe how China presents unique opportunities to develop new environmental indicator systems for measuring and managing CE.

CEs but Incomplete Indicators

A CE is an industrial system focused on closing the loop for material and energy flows and contributing to long-term sustainability (3). CE incorporates policies and strategies for more efficient energy, materials, and water consumption, while emitting minimal waste into the environment (4).

Germany and Japan were pioneers in CE-like policies. Germany's 1996 CE Law sought to reduce land use for waste disposal by focusing on solid waste avoidance and closed-loop recycling. In 2000, Japan's "Sound Material-Cycle Society" focused on solid waste management, land scarcity, and resource depletion because of concerns about shortages of landfill spaces and revitalizing local stagnating industries (5). CE in Japan includes "ecotowns" aimed at reducing landfill requirements (6) and product-specific recycling targets for waste categories to be reached through product stewardship schemes, levies, and voluntary regulatory initiatives for producers and consumers.

China's CE borrowed from Germany, Japan, the European Union (EU), and the United States by incorporating elements of take-back regulations, resource efficiency

goals, reduction goals, and eco-industrial parks (4). But China's socioeconomic environment provides a context different from that of other nations, making it an ideal laboratory for new, expanded CE policies. For instance, Japan's effort focuses on redeveloping stagnating industries and Germany's on waste-management goals, whereas China's CE is a broader systemic policy, more integrated at the national level to include development planning and requiring collaboration by numerous government agencies. These agencies are considering linking CE to China's low-carbon strategy (4).

China's CE has rapidly evolved. Its latest CE promotion law, adopted in 2009 (4), has gained recent political traction. National plans for safe urban municipal solid waste treatment (7), energy saving, and emissions reduction (8) are being implemented on the basis of CE principles. Government agencies are developing tax policies supporting resource recovery in industrial practices. Billions of dollars are being invested in CE-oriented pilot projects, from applications of clean production techniques in specific sectors to municipal and regional eco-industrial development.

Well-designed indicators are valuable for managing environmental development and providing guidelines to improve CE policies. Performance indicators for regions and industrial parks have been developed, based on well-known assessment methods: energy, material flow analysis (MFA), life-cycle analysis (LCA), CO₂ emissions, and economic returns (4). In spite of their usefulness, these indicators may not optimally fit CE assessment needs because they were not originally designed for the systemic, closed-loop, feedback features that characterize CE. Some disregard flow quality and characteristics and the complexity of interactions between the natural environment and socioeconomic systems (9).

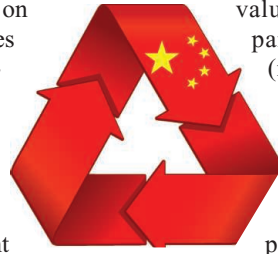
Other indicators of eco-efficiency—carbon and ecological footprints, LCA, economic and energy valuation—mainly focus on individual parameters. Although useful at the local scale of specific processes or products, this specificity is unlikely to provide a

complete picture for managing CE policy. Unidimensional indicators (i) focus on individual aspects of resource use and system metabolism—such as commercial energy demand, emissions, or economic value—often disregarding other parameters and driving forces; (ii) do not account for local ecosystem services or the value of existing natural capital, other than in monetary terms [e.g., (10, 11)], with incomplete assessments leading officials to pay less heed to protecting local ecosystems; (iii) call for policies optimizing an individual resource or flow, thus are less suitable to track diverse, non-linear interactions between human society and the natural system in which economic processes are embedded; and (iv) lack the ability to address waste and emission management, reuse, and recycle strategies that characterize CE.

This suitability gap is more evident at the national and macroeconomic level. Although many indicators prove effective at the scale of specific products and processes, they are limited when considering the broader context and network of CE resource flows. The more complete role of natural systems as a source, sink, and regulator is missing.

An Emergy Indicator System

Given CE's broad systemic aspects, monitoring can be enhanced through emergy-based indicators, a set of environmental accounting indices and ratios (12, 13) capable of capturing both resource generation (upstream) and product (downstream) dimensions. Rooted in ecology, thermodynamics, and general systems theory, emergy is the sum of all available energy inputs directly or indirectly required by a process to generate a product (12). Emergy assigns value to nature's environmental effort and investment (e.g., solar, deep geothermal heat, and gravity) to make and support flows, materials, and services and to contribute to the economic system. Given that solar energy is the dominant energy input to Earth, emergy expresses all inputs and flows in solar-equivalent Joules (seJ), a critical feature that enables distinctions between qualities of resources



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that are not possible under other indicator systems based on user-side human preference values. For example, in energy analysis, a MJ from wood and a MJ from oil contribute the same amount to energy intensity indicators, yet wood and oil derive from different production patterns and generation times within natural cycles, requiring different amounts of solar energy [for photosynthesis (wood) and to convert biomass to fuel (oil)].

The ratio of emergy (seJ) required to make an amount of product (J) is defined as “transformity” (seJ/J) (13), a measure of production cost on the spatial and time scales of the biosphere. For example, production of one joule of electricity might require 2.5 J of natural gas, production of which might in turn require the equivalent of 170,000 J of sunlight. When adding the emergy of machinery, chemicals, labor, and environmental services, 1 J of electricity may require ~300,000 seJ. Flows of renewable, nonrenewable, local, and imported emergy resources (e.g., biomass, coal, or human labor) are ultimately used to calculate environmentally based, systemic performance indicators.

An emergy indicator system includes (i) intensity indicators that target convergence of resources per unit of product, of labor expended, of Gross Domestic Product (GDP) generated, of land developed, etc., and (ii) performance indicators, such as emergy yield ratio (emergy return on emergy investment), emergy loading ratio (a measure of carrying capacity), emergy density (emergy use per unit of time and area), emergy sustainability indicator (an aggregated measure of yield and environmental pressure), emergy investment ratio (emergy investment from outside for a local resource exploitation), and fraction of emergy that is renewable, among others, all of which support multiple performance aspects in resource use.

By accounting for quantity and quality of input flows, keeping track of interactions among system components across scales, and identifying environmental costs and savings of loop-closing strategies at all levels, emergy provides a systemic framework for assessing the performance and sustainability of CE, as well as specific CE implementation processes.

Emergy evaluation has been criticized for implicitly assuming that input resources can substitute for each other (14). But the “quality” feature embodied in the transformity concept (e.g., a joule of fuel is not the same, in environmental cost and functional terms, as a joule of Sun or a joule of electricity) weakens this argument. Practical capabilities and capacities of emergy analysis have been demonstrated in several large-

scale regional analyses [e.g., (15)].

Given CE’s scale, use of an emergy approach does not exclude use of other indicators for their specific purposes, boundaries, and scales but, instead, provides a framework for integration of approaches (16). LCA can effectively measure downstream environmental burden, e.g., the impact of emissions in the production chain. Emergy analysis accurately measures commercial energy cost of a product. MFA can measure mass degradation in a process. Emergy-inclusive CE indicators provide several characteristics that can be integrated with other evaluation methods:

(i) The emergy “supply-side” evaluation system focuses on nature’s investment, on the work performed by the biosphere to generate resources and services, not only the economic value or the mass of resources supplied to the economic system (17). The latter often ignores contributions of ecosystems to economic development (18).

(ii) Perverse methods of allocating environmental burden may cause conflicts between regions (e.g., with burdens and benefits of coal extraction, energy consumption, and emissions unevenly distributed across coal-extracting and coal-consuming regions). A supply-side, emergy-based indicator approach helps track the entire “production cost.” Broadened accounting of whole supply-chain burdens assigns environmental impacts more fairly and discourages inefficient and unnecessary resource depletion.

(iii) Emergy indicators reflect the space, time, and natural activities needed for resource production, which a CE cannot ignore.

(iv) A CE aims to mimic natural patterns, where resources are routinely recycled, reused, converted, upgraded, and stored for future use. In so doing, resources are not depleted, and waste does not accumulate. The emergy method quantifies both the direct and indirect environmental costs of waste management and untreated waste disposal, as well as the advantage of recycling in closed loops (19).

Toward a CE-Oriented Indicator System

Chinese researchers are studying emergy indicators for a large number of CE systems, but these studies are not well organized or unified. A national research committee on integrative environmental performance indicators should be established. Additional research on emergy indicators’ integration with other tools is also needed. Planning and management mechanisms are needed to help determine how research on environmental performance indicators can proceed, with results shared among researchers and translated into practice. Databases and training opportunities

at all levels are necessary. Dispersed international research efforts need convergence toward emergy as a useful policy-making instrument. In addition to the Chinese Academy of Sciences and Natural Science Foundation of China, the U.S. Environmental Protection Agency, EU, and the Italian National Agency for New Technologies, Energy and Sustainable Economic Development are pursuing projects to evaluate emergy’s assessment capability. National governmental agencies (joint agencies in China, such as the Ministry of Environmental Protection and the Natural Science Foundation of China) or international groups, such as the United Nations and International Standards Organization, can provide avenues and repositories for CE-level emergy databases and resources to help emergy become a practical policy tool.

The use of more scientifically supportive and comprehensive environmental measures will aid the legitimacy of economic and environmental decisions concerning resource use and trade. Facing community protests and pressures from nongovernmental organizations related to environmental issues, the Chinese government must strike a difficult balance between scientific evidence and political expediency if their CE and national development effort is to be successful.

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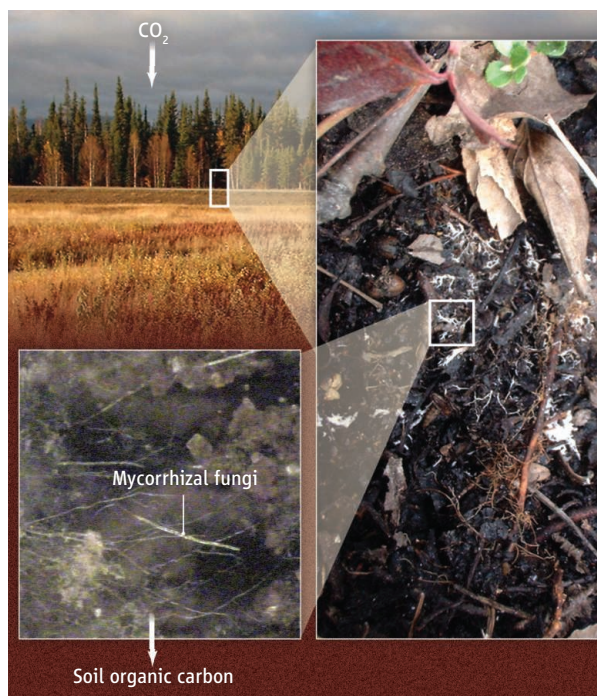
Fungal Carbon Sequestration

Kathleen K. Treseder and Sandra R. Holden

Many soil fungi consist of small, delicate hyphae that permeate a complex matrix of soil particles (see the figure). They are easily damaged, making it difficult to directly observe their activities under undisturbed conditions. On page 1615 of this issue, Clemmensen *et al.* (1) use isotopic and molecular techniques to infer that a common group of fungi, the mycorrhizal fungi, can sequester carbon in the soil. This is important because carbon stored in soil over long periods can help to offset the release of greenhouse gases to the atmosphere. Most fungal species act as decomposers that elicit a net release of CO₂ to the atmosphere, but mycorrhizal fungi could be a notable exception.

Mycorrhizal fungi form symbioses with most plant roots, helping the plants to take up nutrients from soil. As a result, plants that are colonized by these fungi often grow much faster (2). Essentially, the fungi augment the removal of atmospheric CO₂ by their plant hosts (see the figure). A portion of that carbon is then allocated to the mycorrhizal fungi, which use it to build hyphae that extend into the soil (3). Once these hyphae die, the carbon in their tissues could be quickly decomposed by other soil microbes, or it could remain in the soil for years to decades. The longer the mycorrhizal carbon remains in the soil, the greater the potential contribution to soil carbon sequestration. Thus, it is critical to understand the fate of mycorrhizal carbon in ecosystems.

To address this issue, Clemmensen *et al.* investigated a set of boreal forest islands that differ in their wildfire history and soil carbon accumulation. They used a mathematical model to partition soil carbon stocks into carbon derived from aboveground plant litter or from roots and root-associated fungi. This modeling approach revealed that as much as 70% of soil carbon was root-derived, particularly deeper in the soil where root densities were highest. The model findings were corroborated by molecular analyses, which showed that mycorrhizal and other root-associated fungi dominated deeper soils, whereas decomposer fungi were only abundant in



Mycorrhizal fungi associated with plant roots may contribute to carbon sequestration in soils.

The role of mycorrhizal fungi. Plants remove carbon dioxide from the atmosphere during photosynthesis and direct a portion of this carbon belowground to roots, where it is transferred to mycorrhizal fungi. The fungi then incorporate some of this carbon into hyphae. Once hyphae die and decompose, residues of the carbon are converted to organic material in the soil. Clemmensen *et al.* suggest that this process may contribute to long-term carbon storage in soils. Mycorrhizal fungi are the white filamentous structures in both soil photos.

ectomycorrhizal fungi, are especially common in high-latitude systems (8). They dominated the deeper soils in the study by Clemmensen *et al.* At least some of these fungi respond to elements of global change. For instance,

ectomycorrhizal fungi tend to proliferate when exposed to elevated concentrations of atmospheric CO₂ and decline after nitrogen enrichment (which is common in ecosystems surrounding urban and agricultural areas) (7). Any ectomycorrhizal contributions to carbon sequestration could change in concert.

In northern forests, wildfires may also alter the ability of mycorrhizal fungi to sequester carbon in soil on short and long time scales. Wildfires decrease the abundance of ectomycorrhizal fungi, and reduced fungal abundance after wildfires can persist for several years (9). On millennial time scales, Clemmensen *et al.* found that mycorrhizal fungi make smaller contributions to soil carbon in more frequently burned boreal forests, potentially because dead fungal tissues decay faster in these soils. Given that climate warming is likely to increase the occurrence of wildfires in northern forests (10), mycorrhizal contributions to soil carbon sequestration may decline in these regions.

Many questions remain regarding the influence of mycorrhizal fungi on carbon cycling within ecosystems. For example, it remains to be shown whether certain residues of ericoid or ectomycorrhizal fungi consistently contribute to soil carbon storage. The

shallow soils. Furthermore, stable isotope signatures of soil organic matter most closely resembled that of mycorrhizal fungi.

These findings highlight the central role of mycorrhizal fungi in soil carbon sequestration in boreal forests. Historically, ecosystem ecologists have focused on how quickly the carbon in dead plant leaves is converted to CO₂ by decomposers. Yet recent work indicates that the carbon compounds that remain in the soil over the long term have been produced by fungi and other microbes, not by plants (4). These microbially derived compounds are extremely diverse and are thus difficult for decomposers to target (5). They can include remnants of cell walls, such as chitin, glucans, peptidoglycans, or polysaccharides (6). Microbial residues react with one another and with other components of the soil to form materials that cannot be easily converted to CO₂. It makes sense that organic compounds produced by mycorrhizal fungi would fit this scenario.

Mycorrhizal fungi are a dominant component of the microbial community in soils. Changes in their abundance (and hence in their contribution to carbon sequestration) could therefore have global consequences (7). Two groups of mycorrhizal fungi, ericoid and

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glycoprotein glomalin produced by arbuscular mycorrhizal fungi (another major group) is thought to reside for decades in the soil (11). It is not clear whether analogous compounds are constructed by other mycorrhizal fungi and under what conditions. Another open question is whether ericoid and ectomycorrhizal fungi conduct decomposition themselves. Many members of these groups have the physiological capacity to break down and take up soil organic material (8), which could ultimately result in the production of CO₂.

Finally, the extent to which mycorrhizal fungi improve plant growth can also determine how much carbon is deposited in the soil via dead plant material. It is the sum of these three processes—deposition of mycorrhizal residues, decomposition by mycorrhizal fungi, and augmentation of plant growth—that determines how mycorrhizal fungi affect carbon storage.

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BIOCHEMISTRY

A Protease for the Ages

Susan Michaelis¹ and Christine A. Hrycyna²

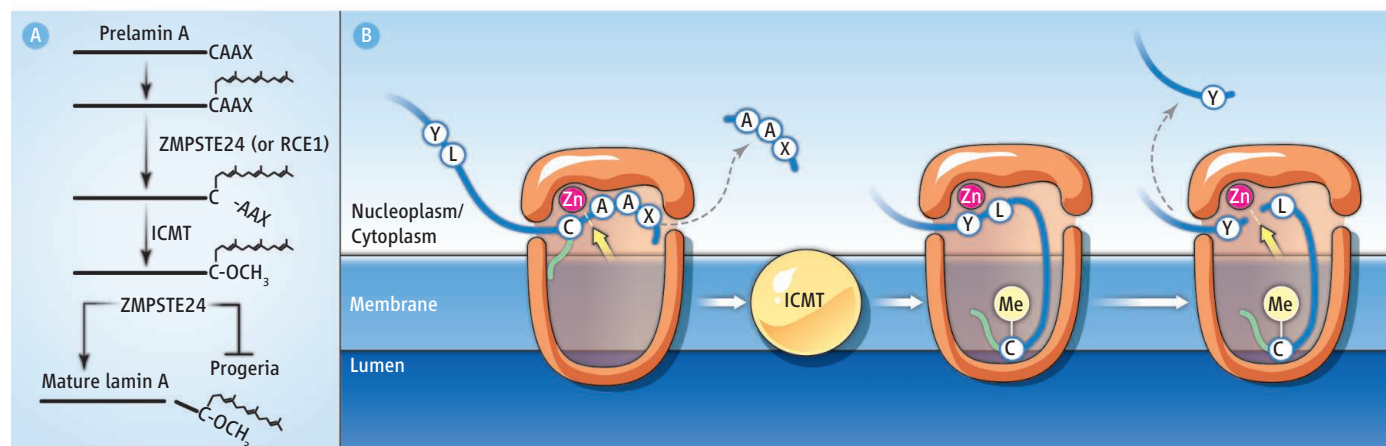
Mutations in the genes encoding the nuclear scaffold protein lamin A or the zinc metalloprotease ZMPSTE24 cause the devastating premature aging disorder Hutchinson-Guilford progeria syndrome (HGPS) and the related progeroid disorders restrictive dermopathy (RD) and mandibuloacral dysplasia (MAD-B) (1–4). Children with HGPS, for example, manifest accelerated aging symptoms, including failure to thrive, hair loss, joint ailments, lipodystrophy, and cardiovascular disease, typically dying from the latter in their mid-teens. In all of these progeroid disorders, a persis-

tently farnesylated and methylated form of lamin A is the “molecular culprit,” exerting dominant-negative effects that promote aging-related symptoms (1). On pages 1604 and 1600 of this issue, Quigley *et al.* (5) and Pryor *et al.* (6) report the three-dimensional crystal structures of the human zinc metalloprotease ZMPSTE24 and its yeast homolog, Ste24p. These proteases play critical roles in two steps of the posttranslational maturation of human lamin A and the yeast mating pheromone α -factor, respectively (7–9). ZMPSTE24 and Ste24p are multispanning membrane proteins and as such, determining their structures by x-ray crystallography represents a substantial accomplishment. The structures should lead to a better understanding of how these enzymes function and how they are associated with aging.

Structures of membrane metalloproteases provide the basis for understanding mutations associated with premature aging.

The ZMPSTE24 substrate lamin A maintains the structural integrity of the nucleus. It is synthesized as a precursor, prelamin A, that terminates in a C-terminal CAAX motif (where C is cysteine, A is generally an aliphatic amino acid, and X is any residue). Like all CAAX proteins, prelamin A undergoes three sequential posttranslational modifications including isoprenylation of cysteine with a farnesyl lipid moiety, endoproteolytic removal of the -AAX peptide by ZMPSTE24 (or by RCE1), and carboxyl methylation (1, 2) (see the figure). Unlike most other CAAX proteins, however, prelamin A undergoes a second cleavage event, also mediated by ZMPSTE24, to yield mature lamin A. This second cleavage removes the last 15 amino acids of the protein, including the newly modified C terminus (4, 7–9). The

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In the hollow. (A) In the posttranslational modification of lamin A, the zinc metalloprotease ZMPSTE24 (or RCE1) mediates processing of the -CAAX motif; ZMPSTE24 then makes a second cleavage. (B) ZMPSTE24 is a membrane protein with a large hollow barrel-shaped chamber enclosing the active site. In the hypothetical reaction scheme shown, the C terminus of lamin A (blue), which has

been modified with a farnesyl moiety (green line), enters the cavity through a gap in the chamber wall between two transmembrane spans, and aligns in the Zn²⁺ active site. ZMPSTE24 removes the -AAX peptide. The membrane protein ICMT then methylates (Me) lamin A. This is followed by removal of the modified C terminus by ZMPSTE24 and the release of lamin A.

ZMPSTE24 structure reported by Quigley *et al.* sets the stage for determining how this protease recognizes two completely different cleavage sites within lamin A.

Although it remains a mystery as to why cells go to the trouble of modifying the C-terminus of lamin A only to have ZMPSTE24 remove it, the finding highlights its role in aging disorders and suggests a potentially important role for the metalloprotease in normal human aging. It will be critical to ensure that new protease inhibitors and other drug types do not inadvertently block ZMPSTE24 through unintended off-target interactions. Already, an unexpected *in vitro* inhibition of ZMPSTE24 by several HIV protease inhibitors has been shown that could possibly contribute to the side effects of HIV treatment (10).

The most surprising result from the essentially superimposable crystal structures of human ZMPSTE24 and yeast Ste24p is the presence of a voluminous membrane-enclosed hollow chamber, not previously seen in a membrane protein, which could serve as an enzymatic reaction vessel. This barrel-shaped chamber, formed by seven transmembrane segments and capped at both ends, is estimated by Pryor *et al.* to be large enough to accommodate ~450 water molecules or a ~10-kD protein. The structures show that the active site of these zinc metalloproteases faces the interior of the chamber at the membrane interface. Thus, after a prenylated substrate enters the chamber, it is ideally poised for proteolysis. Interestingly, the

structures illustrate how several MAD-B and RD disease mutations that block enzymatic activity (11) could affect the zinc metalloprotease active site or access to it.

The voluminous hollow chamber is far larger than needed simply for catalysis, presenting a challenging puzzle as to its function. One possibility is that the chamber sequesters the farnesylated 15-amino acid lamin A tail generated in the final proteolytic step of prelamin A processing. Persistent farnesylation and methylation of prelamin A is implicated in disease states. Thus, it is conceivable that the cleaved peptide itself may confer similar phenotypes if allowed to freely diffuse within the membrane. Sequestration and possibly further proteolysis of the peptide in the chamber might neutralize such potentially toxic effects.

The ZMPSTE24 and Ste24p structures also begin to provide clues as to how substrates undergo dual cleavage. The C-terminal farnesylated portion of the substrate could insert in the active-site cavity through a gap in the chamber wall between two transmembrane helices. After proteolysis of the -AAX sequence and prior to the second cleavage, the farnesylated cysteine on the substrate is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT). It has been assumed that ZMPSTE24/Ste24p releases the substrate and then rebinds it after methylation. However, the structures raise the intriguing possibility of a processive mechanism, in which the substrate never completely leaves the chamber. In this model, after the

first cleavage, the substrate would move through the enzyme, project outward through another gap between membrane spans, undergo methylation by ICMT (itself an integral membrane enzyme), and then retract back into the chamber for the final cleavage. Regardless of the mechanism, the roles of the farnesyl and methyl groups in positioning the substrate for the cleavage by ZMPSTE24/Ste24p remain an open question.

Ultimately, determining how ZMPSTE24 functions and how to avoid accidentally interfering with its function will have important implications for pharmaceutical drug design. This knowledge will also widen our understanding of premature aging diseases and normal physiological aging.

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CHEMISTRY

FRETting over the Spectroscopic Ruler

Jay R. Winkler

An excited fluorescent molecule may not emit light if it can transfer its excitation energy to a nearby molecule. Förster's theory describing electronic excitation energy transfer (EET) (1) is now 65 years old, but it is not ready for retirement. It still enjoys popularity through the FRET (2) method for determining molecular-scale distances. A seminal paper by

Stryer and Haugland in 1967 provided experimental support for the predicted inverse sixth power distance dependence of FRET efficiency and coined the term "spectroscopic ruler" (2). Despite the widespread use of fluorescence spectroscopy to determine distances between a "donor" (D) and "acceptor" (A), the method can be tricky, and numerous caveats concerning the FRET model have appeared. On page 1586 of this issue, Consani *et al.* (3) point out another, based on their use of two-dimensional ultraviolet (UV) transient spectroscopy to dis-

entangle the competing contributions of electron transfer and FRET to the decay of excited tryptophan (Trp) residues in myoglobin (Mb).

Förster's elegant derivation (1) began with a golden rule expression for the FRET transition rate. The electronic matrix element described the Coulombic interaction between electrons on D and A. In the limit where D and A are separated by a distance R_{DA} that is substantially greater than the dimensions of D and A, the Coulombic potential can be approximated by the inter-

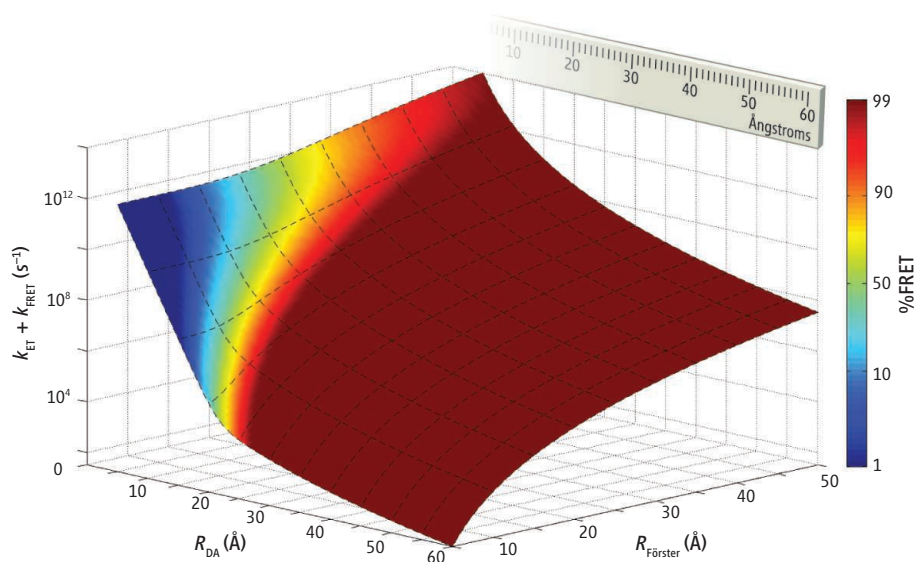
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action energy of two electric dipoles, and the EET rate will vary as R_{DA}^{-6} . The directions of the D and A transition dipoles are critical for determining the EET probability; but in most cases, orientational averaging is assumed. Förster linked the electronic matrix elements to the overlap of the D fluorescence and A absorption spectra, allowing the transfer rate to be calculated from readily measured quantities. The orientation factor, spectral overlap integral, and donor quantum yield for a D-A pair are frequently subsumed into the Förster radius, the distance at which EET is 50% efficient.

Five years later, Dexter's inclusion of antisymmetrized wave functions (4) led to the recognition that electron exchange interactions contribute to EET at small values of R_{DA} . The eponymous Dexter EET mechanism, which can be viewed as double electron transfer (5), requires overlap of D and A wave functions, decays exponentially with distance, and can involve spin-forbidden transitions. The short range of the interaction relegates exchange energy transfer, in most cases, to collisional processes.

Stryer and Haugland calibrated the spectroscopic ruler with D and A separated by poly(L-proline) oligomers (2). Experiments indicated that the polypeptides adopted a trans proline type II helix structure in oligomers of 5 to 12 residues; structural models allowed them to correlate FRET efficiency with R_{DA} . More recent single-molecule FRET measurements along poly(L-proline) helices reveal that peptides of the length used by Stryer and Haugland can be approximated by a rigid rod, but longer proline oligomers are decidedly more flexible, displaying persistence lengths on the order of 40 Å (6). Had Stryer and Haugland used longer peptides and a FRET pair with a greater Förster radius, they might not have found such faithful agreement with theory.

Excited molecules can decay by several nonradiative pathways besides EET, with single electron transfer (ET) foremost among them. Like FRET, ET is described by an extraordinarily robust theory that has survived more than a half-century of experimental scrutiny (7). Work with proteins has shown that ET rates depend exponentially on R_{DA} ; a decay factor of 1.1 Å^{-1} describes most systems (8). This steep distance dependence limits single-step excited-state ET reactions to distances under 20 Å (see the figure), but multistep reactions can transport charges over distances of 30 Å or more (9). The exponential dependence would put ET on par with Dexter EET were it not for the twofold-greater



How reliable a ruler? In the competition between electron transfer (rate constant k_{ET}) and Förster resonance energy transfer (rate constant k_{FRET}), shorter donor-acceptor separations (R_{DA}) and smaller Förster radii ($R_{\text{Förster}}$) tend to favor the transport of electrons between sites (blue to green zones). For distances greater than 20 Å, however, FRET is expected to be the dominant process for transferring energy (red areas). The plot was generated assuming driving-force optimized ET at 295 K and a 10-ns excited-state lifetime for D (1, 7, 8).

distance decay factor expected for the latter process (5).

FRET is especially useful for studying proteins but, aside from those found in the photosynthetic machinery, nature has provided sparingly few chromophores for distance measurements. A favorite fluorophore is Trp, despite its rather problematic photophysical properties (10), and iron-porphyrins provide a convenient built-in acceptor for studies involving heme proteins. The fluorescence from Trp⁷, and Trp¹⁴ in horse ferric Mb, is heavily quenched by a process long-believed to be FRET (11). Consani *et al.* examined UV transient absorption spectra as a function of the excitation laser wavelength to delineate Trp decay routes in Mb. They report that, although Trp⁷ decay (time constant $\tau \sim 140$ ps) is consistent with FRET, ET to the heme dominates deactivation of excited Trp¹⁴ ($\tau \sim 20$ ps).

Their observations are consistent with the relative Trp-heme distances (Trp⁷, 21.2 Å; Trp¹⁴ 15.2 Å) and the shorter ET interaction zone (see the figure). Still, R_{DA} alone does not tell the full story: A prior report from Chergui's lab indicates that Trp⁵⁹-to-heme EET in cytochrome c proceeds in ~ 700 fs ($R_{\text{DA}} = 9.7$ Å) without any apparent contribution from ET (12). Naïve consideration of excited Trp decay in Mb would have implicated ET as the primary Trp⁵⁹ decay pathway in cytochrome c.

Remarkable advances in time resolution and complexity of laser spectroscopy,

along with parallel progress in theoretical and computational modeling, are providing an increasingly clearer picture of electronic excited-state deactivation processes (13). After more than six decades, the spectroscopic ruler remains a reliable gauge in the chemist's toolbox, despite the great difficulty in reading the markings on the short end of the scale.

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ECOLOGY

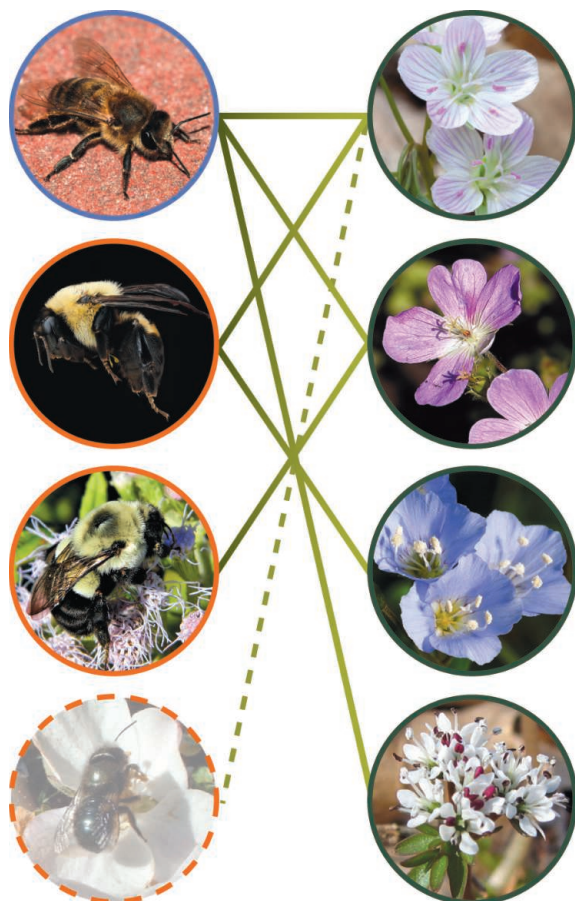
The Global Plight of Pollinators

Jason M. Tylianakis

Three-quarters of global food crops depend at least partly on pollination by animals, usually insects (1). These crops form an increasing fraction of global food demand (2). Given this importance, widespread declines in pollinator diversity (3) have led to concern about a global “pollination crisis” (4). However, others have argued that this concern is premature and that conservation action cannot yet be justified on the basis of deteriorating pollination (5). Are concerns of a pollinator crisis exaggerated, and can we make do with better management of honeybee colonies? Two articles in this issue provide compelling answers to these questions. On page 1611, Burkle *et al.* demonstrate that native wild pollinators are declining (6). On page 1608, Garibaldi *et al.* show that managed honeybees cannot compensate for this loss (7).

The arguments against a pollination crisis are based on the number of staple crops (such as rice, maize, and wheat) that do not require animal pollination. Furthermore, it has been questioned whether pollination is actually declining globally. If pollinators do decline or go extinct, other generalist species may be able to fill the gap (5), assisted by domesticated honeybees, which are increasing in numbers globally despite declines in certain regions (2). Do these arguments hold up? Can we get by with just honeybees?

To measure the extent to which environmental changes over the past 120 years have disrupted plant-pollinator interactions, Burkle *et al.* revisited sites in Illinois in 2010/2011 that were sampled in the late 1800s and in the 1970s. They found that



The role of wild pollinators. Schematic of a plant-pollinator network such as that studied by Burkle *et al.* (6). Circles depict plant or pollinator species. Each solid line represents an interaction between a plant and a pollinator. Dashed lines and circles represent species and interactions that have gone extinct. Garibaldi *et al.* (7) found that the honeybee (blue circle) was less effective than many of its wild pollinator counterparts (orange circles).

half of the bee species present historically were absent and that less than one-quarter of the historical plant-pollinator interactions were still observed. Moreover, the quantity and quality of pollination experienced by plants has also declined. The herbaceous perennial flower *Claytonia virginica* now receives a quarter of the pollinator visits it received in the 1970s. Those pollinators that do still visit are less faithful to that species (that is, they carry pollen from many other plants), which can negatively affect pollination success.

Using a network approach to study plant-pollinator interactions (see the figure), the

Wild pollinators are in decline, and managed honeybees cannot compensate for their loss.

authors found changes that suggest that overall pollination will be less resistant to extinction in the future. Present-day interactions not recorded in the historical samples tended to involve species with historically narrow diets. This contrasts with the concept of preferential attachment in networks, whereby highly connected species should be more likely to acquire new interactions with others. The opposite finding by Burkle *et al.* (6) may be explained by changes to pollinator and plant phenology (8) and suggests that even seemingly specialist species may have an important role in filling the pollination gap after extinctions. Burkle *et al.* also found that species loss was nonrandom, such that specialists, parasites, cavity-nesters, and species that participated in weak historic interactions were most likely to go extinct. This result, along with recently discovered nonrandomness in the loss of pollinator interactions in fragmented habitats (9), foreshadows a systematic alteration of global pollination networks under a suite of environmental changes.

From a food production standpoint, the decline of wild pollinators could be ignored if honeybees can do the same job. It has even been suggested that honeybees can do the job better (10, 11). If this were true, then we should focus all our efforts on protecting honeybees and invest as much as possible in combating colony collapse disorder, Varroa mite, and any other threats to the species charged with protecting global food security (11).

However, the landmark study by Garibaldi *et al.* suggests that putting our hopes and efforts into honeybees may not yield the desired results. The authors examined pollination of 41 crop systems from 600 field sites on every continent except Antarctica. They found that, even though honeybees frequently deposit a lot of pollen, they apparently do so ineffectively. The percentage of flowers that produced fruit was relatively low when flowers were visited by honeybees, and increased visitation by honeybees only increased fruit production in 14% of the systems surveyed. In contrast, the increase in fruit production after visitation of flowers by wild insects was twice as great as that produced by honeybees, and flowers pollinated by wild insects were more consistent

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in their fruit production. This pattern was generally consistent across a great variety of the most important pollination-dependent crops. The benefit associated with wild bees did not depend on whether or not honeybees were present. Thus, conservation of wild bee diversity will be paramount even when managed honeybees are used.

The two studies (6, 7) highlight the effects of environmental change on pollinator-plant interactions and the risks of putting all our eggs in one basket for pollination. Garibaldi *et al.*'s finding that fruit set increased and became less variable with pollinator diversity, independently of visitation by honeybees, highlights the importance of in situ bio-

diversity for food production. This challenges the validity of land-sparing conservation approaches (12), which advocate the protection of biodiversity only outside farmed areas, and the further intensification of agricultural land use. Above all, the studies show conclusively that biodiversity has a direct measurable value for food production and that a few managed species cannot compensate for the biodiversity on which we depend.

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COMPUTER SCIENCE

Toward a Green Internet

Diego Reforgiato Recupero

Information and communication technology (ICT) has been extensively used to monitor energy use in a variety of applications. However, the use of ICT itself has led to huge increases in energy consumption. Today, we are witnessing a rise of energy costs, customer increase, more on-demand services using cloud architectures, mobile Internet, a diffusion of broadband access, and a growing number of services offered by internet service providers (ISP). Consequently, energy efficiency is quickly becoming a high-priority issue for the Internet.

Telecom companies such as Telecom Italia used over 2 terawatt hours (TWh) in 2006 (about 1% of the entire Italian energy demand), increasing by ~8% compared with 2005 and ~12% in 2004 (1, 2). Comparable numbers were reported by Telecom France and British Telecom, by Verizon in the United States, and by NTT in Japan. In Germany, 20% of Internet energy usage was due to cooling systems. In 2005, European Internet operators had an overall network energy requirement equal to 14 TWh, increasing to 21 TWh in 2010, and projected to rise to 36 TWh in 2020 if no green network technologies are embraced. Moreover, the world's data centers consumed over 270 TWh in 2012; it is estimated that they will consume 19% more energy in the next 12 months than they have in the past year (3). The cost of new equip-

ment has been overtaken by the cost of the required power and cooling infrastructure and will soon be exceeded by the lifetime energy costs (4).

Although Internet traffic volume doubles every 3 years, the increase in usage has not been matched by a similar increase in network energy efficiency. Current networks, devices, links, and data centers are provisioned with hardware and software designed for peak loads that do not include any power

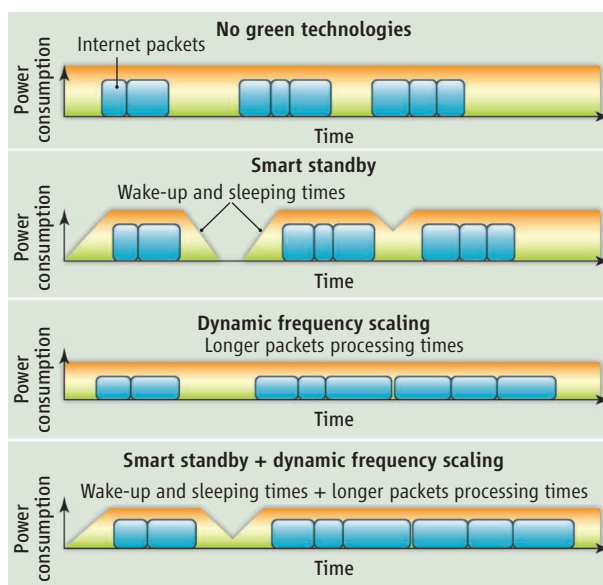
Methods for energy efficiency savings will be needed to meet the growing demands of increasing Internet usage.

management capabilities. As a consequence, the overall power consumption remains more or less constant for differing Internet traffic levels even while peak loads rarely occur.

As the Internet evolves, it is apparent that energy efficiency needs to be addressed.

Over the past 3 years, a number of international research projects (5–7) have been initiated, with specific efforts including methods to redesign the power management features of network devices to improve efficiency (8, 9). Two of the most exciting new techniques are smart standby (10) and dynamic frequency scaling (also known as CPU throttling). The former will allow unused parts of a network device to be put into very low power states, where only very basic functionalities are performed. This method is key for reducing energy consumption because it will allow switching some portion of the network to a sleep mode in a smart and effective way.

Dynamic frequency scaling allows us to trade off the energy consumption and processing capacity of internal blocks while satisfying the current traffic load and quality of service constraints. This ensures that when the



Improving efficiency. Packet service times and power consumption when no green technologies are applied, with only smart standby, with only dynamic frequency scaling, and with both smart standby and dynamic frequency scaling.

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system is under partial load, parts of it can be throttled to decrease the power consumption without a reduction of overall performance.

The power consumption and the packet service times can be depicted for different scenarios (see the figure). When smart standby is used, different idle states are usually designed by selectively turning off an increasing number of hardware units. This leads to a reduction of the energy consumption during idle times; however, longer times are needed to wake up all the hardware. Similarly, dynamic frequency scaling hardware support is designed by pre-selecting a set of operating clock frequencies whose values are submultiple of the maximum one and that provide silicon stability. Dynamic frequency scaling causes a stretching of packet service times, while the sole adoption of smart standby introduces an additional delay in packet service, due to the wake-up times. Finally, the joint adoption of both energy-aware capabilities may not lead to outstanding energy gains because dynamic frequency scaling causes larger packet service times and, consequently, shorter idle periods.

The next generation of network devices will include local control policies (8) that will be able to set up and synchronize their energy-aware capabilities. For example, analytical models have been defined to allow designers of green network devices to calcu-

late in advance the temperature statistics of a device and decrease it when possible (11). Reduction of the average temperature allows designers to reduce the hardware size and the size of passive and active cooling systems and thus reduce energy consumption.

New algorithms for network-wide control, both distributed and centralized, are starting to take green metrics into account. For example, a possible distributed solution currently builds upon link-state protocols and puts links in an Internet protocol-based network into sleep mode at appropriate times (12). This method allows limiting the amount of shared information, avoiding explicit coordination among nodes, and reducing the problem complexity. Thus, the switch-off decision takes the current load of links and the history of past decisions into account.

With such practices spreading into industry, large companies are now building energy-efficient data centers for minimizing data-center power costs (13). Moreover, measurement of servers in a production data center from both power and performance reveal that most servers are underused and have similar activity patterns across the days of the week (14).

The fundamental problem of greening the Internet is to strike a fine balance between the demands of performance and the limitations of energy usage. New research initiatives in energy optimization have revealed several

aspects of the Internet that can be streamlined. Addressing the issues of energy efficiency will allow us to draw deeper conclusions on how new network systems can be smarter and more effective.

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NEUROSCIENCE

Neural Stem Cells, Excited

Jenny Hsieh¹ and Jay W. Schneider²

Although the brain is generally considered a terminally differentiated organ, new nerve cells are made every day through a process called “adult neurogenesis,” which occurs in specialized regions like the hippocampal dentate gyrus (1). Stem cells in the brain sample electrical signals (activity) from neighboring neurons, deciding which genes to express and which signaling pathways to launch toward developing their own neuronal identity. Why would stem cells be able to respond to exogenous neuronal electrical activity,

which can be considered a highly specialized function? Indeed, it seems counterintuitive insofar as one of the defining functions of all stem cells is to actively maintain the undifferentiated state.

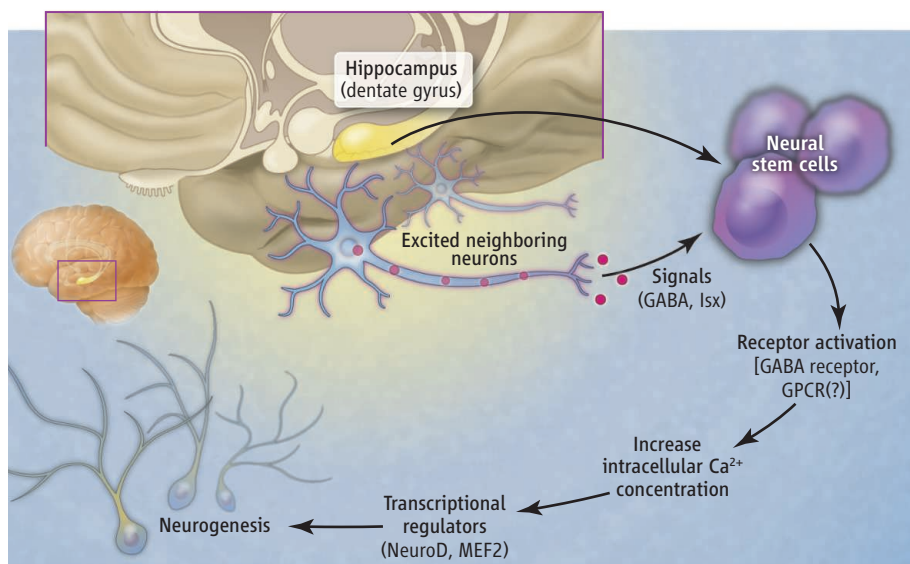
The central feature of brain function is the transmission of electrical signals from neuron to neuron in complex networks and circuits. How neurons “listen and talk” to each other is determined by cell identity—that is, which genes (e.g., encoding receptor systems or neurotransmitter biosynthesis pathways) are expressed by an individual neuron and which are not. The complexity of this information exchange is staggering because billions of neurons, falling into distinct molecular and cellular phenotypes based on their gene expression patterns, are communicating with each other at the same time.

Electrical activity in the adult mammalian brain triggers neurogenesis.

Cultured neural stem/progenitor cells isolated from rodent hippocampus can respond to external neural activity and differentiate into neurons (2). This activity-dependent neurogenesis requires Ca^{2+} channels and receptors for the neurotransmitter *N*-methyl-D-aspartate (NMDA) on proliferating stem/progenitor cells, and hence is called “excitation-neurogenesis coupling.” In vivo, type 2 stem/progenitor cells [expressing nestin, a protein marker for neural stem/progenitor cells; they also morphologically lack projections (dendrites and axons) from the cell body] express receptors for γ -aminobutyric acid (GABA) and can be activated by this chemical when released by nearby active neurons (3, 4). A possible mechanism for excitation-neurogenesis coupling is GABA-mediated depolarization (4), previously

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Exciting development. The differentiation of stem/progenitor cells of the hippocampus can be influenced by the electrical activity of surrounding mature neurons. The pathways are only beginning to be elucidated. Isx, isoxazole; GPCR, G protein-coupled receptor.

described in mouse embryonic neuronal progenitor cells (5). Depolarization causes an increase in intracellular Ca²⁺ concentration similar to that evoked in cultured neural stem/progenitor cells (2). This excitation signal is relayed to the genome wherein the transcription factor NeuroD is rapidly activated to promote neurogenesis (2, 4). Once proliferating stem/progenitor cells differentiate into slightly later stages of neuroblasts and immature neurons, excitation signaling in the form of GABA is also necessary to drive the functional integration of newborn neurons (6, 7).

These early studies highlight the importance of excitation-neurogenesis coupling in the stepwise differentiation and maturation of adult neural stem/progenitor cells in the mammalian brain. They do not, however, address whether all types of neural stem cells in the brain have this response to activity. For example, neural stem cells in the brain called type 1 or radial glial-like (RGL) cells can respond to neural activity. RGLs maintain the adult neural stem cell pool in the hippocampus by remaining quiescent; the maintenance and activation of RGLs is dynamically controlled by experience and aging (8, 9). Nestin-expressing RGLs can be activated by GABA (10). The absence of functional GABA_A receptors results in rapid exit from quiescence and increased production of RGLs. Cells in the stem cell niche required to maintain RGLs are interneurons (expressing the Ca²⁺-binding protein parvalbumin), a source of GABA. Modulation of GABA signaling affects the generation of more RGLs or causes them to remain quiescent, but not to differentiate. Thus, these particular neu-

ral stem cells are sensitive to activity, but have a different behavior. Many open questions remain, including the identity of other cell types that work in concert with parvalbumin-expressing interneurons and RGLs, and whether other possible niche components exist for neural stem cell excitation.

One limitation of assessing the specific function of endogenous chemicals, such as amino acids, peptides, and monoamines, on excitation-neurogenesis coupling is that classical neurotransmitters do not appear to distinguish between stem cells and mature neurons. Theoretically, stem cell-selective chemicals could provide a tool to probe and explore mechanistic pathways of excitation-neurogenesis coupling. Among the small molecules found to activate gene expression exclusively in neural stem/progenitor cells were 3,5-disubstituted isoxazoles (11). This compound class triggered differentiation of cultured neural stem/progenitors and promoted hippocampal adult neurogenesis when administered to mice systemically (12–14). Moreover, isoxazole-9 improved hippocampal-dependent memory formation in adult mice (14). This small molecule causes an increase in intracellular Ca²⁺ concentration through both classical neurotransmitter signaling pathways such as that controlled by the NMDA receptor, and pathways activated by G protein-coupled receptors. The receptor responsible for isoxazole-9's neural progenitor selectivity has not yet been identified, but the compound elicits the expression of differentiation genes that are also turned on in response to the electrical activity of other neurons. This includes the gene encoding

myocyte-specific enhancer factor 2 (MEF2) (see the figure) (12, 14). MEF2 is a critical regulator of muscle development in all muscle lineages, including the heart (15). Studies of excitation-neurogenesis may potentially guide a better understanding of how electrical stimulation and stretch of developing cardiac precursor cells promotes myocyte differentiation and contractile function, especially in the context of cardiac tissue engineering (16).

Generally, stem cells deflect or reject environmental signals (e.g., through drug efflux pumps) that might trigger differentiation and the loss of stemness. In adult tissues and organs, like the brain, stem cells already possess sophisticated receptor systems to immediately sense environmental changes. However, instead of initiating cellular specialization, which would possibly irreversibly deplete the stem cell pool, these receptor systems help stem cells decide how many (or how few) progeny cells to produce. It may be that the neurogenesis response of stem/progenitor cells to activity is an adaptive mechanism to maintain regional homeostasis: increasing stem cell production when local circuitry activity levels are low, and restoring quiescence when activity levels are high.

It is not entirely surprising that the adult brain continues to develop under the influence of electrical activity, such that similar to mechanical stretch regulation of muscle growth, behavior and circuit activity control adult neurogenesis. A potential “cost” of excitable stem cells is inappropriate activation after pathological forms of activity, such as seizures. Understanding how electrical activity controls adult neural stem cell properties and neurogenesis is likely to provide mechanistic insight into neural circuit function and new tools for mapping human brain connections.

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10.1126/science.1237576

IBI* SERIES WINNER

Integrating Inquiry-Based Teaching with Faculty Research

Tadashi Fukami

Biology 44Y, an IBI prize-winning module, helps students do science by practice, with a focus on plant-pollinator-microbe interactions as a model system.

The need for inquiry-based instruction in undergraduate education is well recognized (1, 2), but it has not been adopted widely in place of traditional “cookbook” instruction, where students follow lab manuals to reach known answers (3). One problem hindering the change may be insufficient time and resources for faculty to undertake new ways of teaching when they are under pressure to maintain productive research (3–5). Recently, my colleagues and I argued that one solution might be to build inquiry-based courses on faculty research programs, essentially combining teaching and research as synergistic activities (5). Here, I describe how I became involved in such an effort, in the hope that sharing my experience might help accelerate the spread of inquiry-based instruction.

Before joining the Stanford faculty, I struggled to juggle teaching and research in my first faculty position elsewhere. Because of this experience, the idea of designing teaching and research in ways that make the two mutually beneficial, suggested by Stanford’s Center for Teaching and Learning staff during new faculty training sessions, made an impression on me. I thought of this suggestion when I was later invited to participate in a reform of introductory biology laboratory courses. In response to student feedback in a curriculum assessment, the Biology Department was seeking to turn their two-course laboratory series—Biology 44X, on cellular and molecular biology, and Biology 44Y, on ecology and evolutionary biology—into modern inquiry-based classes, and my task was to design and teach a new Biology 44Y.

To ease the transition, the department concurrently offered, for 2 years, the original cookbook-style course taught by the existing instructional team—consisting of an instructor, 10 graduate students working as teaching assistants (TAs), a coordinator, and a laboratory manager—and the new inquiry-based course taught by a smaller team led by me. Concurrent offering served two purposes. First, implementing the new class with gradu-



Study organisms. *Mimulus aurantiacus* flowers visited by an Anna’s hummingbird, *Calypte anna*. Hummingbirds and other pollinators transport nectar-inhabiting microorganisms from flower to flower as they feed on floral nectar.

ally increased numbers of students allowed us to try various pedagogical methods. Second, the two versions could be simultaneously assessed and directly compared by colleagues at Stanford’s School of Education (6, 7).

In 2010, we had 20 students enrolled in the new course, and they were taught by two TAs and me. In 2011, we expanded the new class to 34 students, and they were taught by an instructor, four TAs, and me. We then went full-scale in 2012, when the entire staff, consisting of three instructors, five TAs, two coordinators, a laboratory manager, and me, joined forces to teach all 132 registered students. Despite the big jump from 34 to 132 students, our experience during the initial 2 years helped us better manage the large class. By 2012, we had a good sense of the logistical challenges that each activity would entail and could plan accordingly. That year, we also made efforts to maintain close communications among the instructional team members. Also, I taught the first lab section of each week so that other instructors could come to observe at least part of the activities to ensure that the multiple sections were consistent and well coordinated for data collection.

Building on one of my research group’s projects, the course focuses on ecological interactions among a species of flowering plants, the hummingbirds and insects that pollinate the plants, and the microorganisms that inhabit the floral nectar of the plants and move from flower to flower by hitchhiking on pollinators (8–10). Our aim is to use these interactions (see the first photo) as a case study for the students to practice as many of the same approaches taken by professional biologists as possible. The primary goal is not to gain specific knowledge of the organisms that the students study, but to develop understanding of how experiments are designed, how data are interpreted, and how the level of certainty in

scientific knowledge can be evaluated. We believe this is an important goal for all students, even those who will not continue in biology, because many environmental, agricultural, and medical issues that are relevant to every citizen today require a high level of scientific literacy.

The course is structured around a 1-hour lecture and/or discussion session and a 4-hour lab or field session each week. Each lab section is guided by an instructor and a TA and contains 14 to 20 students. Stanford’s Jasper Ridge Biological Preserve is our field site, located 15 min from the main campus (8). The students visit the preserve once a week during the lab session by a university bus during 8 of the 10 weeks of the course.

Over the first few weeks, students are introduced to the natural history of the plant-pollinator-microbe interactions. They also review the methods of hypothesis testing by basic statistical analysis, with real examples from peer-reviewed journals used as a guide. After that, each pair of students choose, as the focus of their project, one of the following abiotic factors: light, temperature, and water; and one of the following biotic fac-

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tors: plants, pollinators, and herbivorous insects. Each pair then formulates two or three testable hypotheses regarding the relation between nectar-inhabiting microorganisms and the two factors (one abiotic and one biotic) of their choice. For example, one student pair hypothesized that increased ambient temperature would result in a larger number of flowers on a plant, which would, in turn, increase pollinator visits and microbial abundance in nectar.

For hypothesis testing, students are given a large set of shared data (as detailed in the supplementary materials), most of which we have students collect (see the second photo). The data set provides ample room for each team to ask unique questions and design ways to analyze data, while at the same time increasing the common ground on which peer discussion can occur. At the end of the course, each pair presents results in both a talk and a paper. Throughout, we provide step-by-step guidance on how to frame scientific questions, statistically analyze data, and write a paper in a format appropriate for submission to a peer-reviewed journal (5–7).

Over 3 years of course implementation, it has been rewarding to see mutual benefits to student learning and research effort. Assessment by the Stanford School of Education colleagues indicates that our course is achieving our educational goals (6, 7). Moreover, many of the student projects have yielded findings unknown to science. Use of a system and topic with which the faculty member leading the teaching effort is familiar (8–10) helps to ensure that students are collecting data that contribute to new discoveries. At the same time, students often find new ways to collect and analyze data

About the author



Tadashi Fukami is an Assistant Professor in the Biology Department at Stanford University. He studies ecological community assembly, with a focus on historical contingency, or when and why the structure and function of ecological communities are contingent on the past history of species immigration. This work involves various organisms, including the nectar-inhabiting microbes described here. He received a bachelor's degree from Waseda University, a master's degree from the University of Tokyo, and, in 2003, a Ph.D. from the University of Tennessee, Knoxville. After postdoctoral work at Landcare Research, New Zealand, he was an Assistant Professor at the University of Hawaii at Manoa before moving to Stanford in 2008.

that the instructors have not thought about. One example involves data collection initiated by students on the relationship between nectar pH and microbial abundance. Their results were intriguing and later prompted my research group to integrate nectar pH into our work (9). These and other student-collected data have been used in peer-reviewed papers, in which student contributions are acknowledged (8–10).

We recently made six suggestions for successful integration of teaching and research, including (i) a low barrier of technical expertise for students to collect data, (ii) established checks and balances to ensure that student mistakes will not compromise research quality, (iii) a diverse set of variables that present many choices for students to investigate without overwhelming the instructional team, (iv) a central database into which students can upload data, (v) assessment measures that are

representative of real-world science, and (vi) involvement of instructors with expertise in the study system (5). Here, I add one more to the list: (vii) cultivation of a communal experience among students by keeping lab sections small. Inquiry-based learning is most effective when a small number of students work in a collaborative environment, exchange ideas, and ask interrelated questions. Although challenging for high-enrollment classes like ours, implementing our

suggestions is possible if instructors and TAs that lead small sections receive good training beforehand. For example, we meet for 4 hours weekly for 6 to 7 weeks before the course so that the team can learn the field, laboratory, and statistical methods that the students will use. Overall, my experience leaves me optimistic that inquiry-based instruction can become widespread through integration of teaching with faculty research programs.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6127/1536/DC1

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Data collection. Students in the Biology 44Y class collecting data on pollination at the Jasper Ridge Biological Preserve of Stanford University.



AAAS ANNUAL MEETING

In Boston, S&T Leaders Hail Science as an Economic Driver

The 179th AAAS Annual Meeting drew the world's top scientists and engineers, but the message that repeatedly rallied participants was an economic one: Nations succeed when they persistently support research and development.

R&D can yield a high rate of economic and social return, according to the experts gathered in Boston, but it can be difficult to convince policy-makers to practice the patient investment strategy needed to reap these rewards.

The discussions often sounded like a briefing aimed at U.S. lawmakers, who at the time of the meeting were 2 weeks away from allowing massive federal budget cuts to go into effect, on 1 March. An analysis by AAAS has concluded that these cuts, known as sequestration, could devastate U.S. science by reducing federal R&D spending by about \$54 billion over the next 5 years.

Already, the share of the U.S. economy devoted to R&D expenditures has fallen to 2.77% in 2011, down from 2.84% in 2010

and 2.91% in 2009, according to a January report by the Organization for Economic Cooperation and Development.

In an address marking the end of his AAAS presidential term, William H. Press said that federal funding has picked up the slack from American industry, which has decreased its basic research investments in an effort to remain strong in an increasingly competitive global economy.

"But the danger today, as I see it," Press said, "is that the same kind of decision that was made by corporations, by industry in the 1990s, could be made by countries now."

That could lead to a new zero-sum world, he added, where "no country is willing to invest in the basic research of the future, where all are scrambling to compete over diminishing returns on past investments."

Science's role as an economic driver was a recurring topic at the 14 to 18 February meeting, which convened nearly 10,000 researchers, policy-makers, educators, journalists, and students under the theme "The Beauty and Benefits of Science."

At least two decades' worth of studies support that idea that R&D spending can produce strong economic growth and should be considered an investment. These studies are transforming how countries think about

R&D, said Stephen Merrill, the executive director of the National Academies' Board on Science, Technology, and Economic Policy. The United States' national accounts, he noted in a symposium, have long "treated R&D as an expense without any long-lasting return."

But for some policy-makers, Merrill said, there is a growing shift in perspective "from one of demanding that we demonstrate value for the money, to one of emphasizing that we have to improve value for the money."

European finance ministers are also asking for more data on how investment in scientific innovation can help rebuild the continent's battered economies, said Robert-Jan Smits, the European Commission's Director-General in the Directorate-General for Research and Innovation. He said that European researchers have been especially vocal during negotiations for the new EU research funding program set to launch in 2014. "When we had budget discussions 7 years ago, the farmers, they fought for their agricultural subsidies, and the fishermen for their subsidies by blocking the ports, but the scientists stayed in their labs. This time it was a different story."

Smits spoke at a topical panel with Anne Glover, the European Commission's first chief scientific adviser. Glover said that her position, created in 2011, is "a very strong public signal and commitment that certainly for Europe our future is going to be science, engineering, and technology."

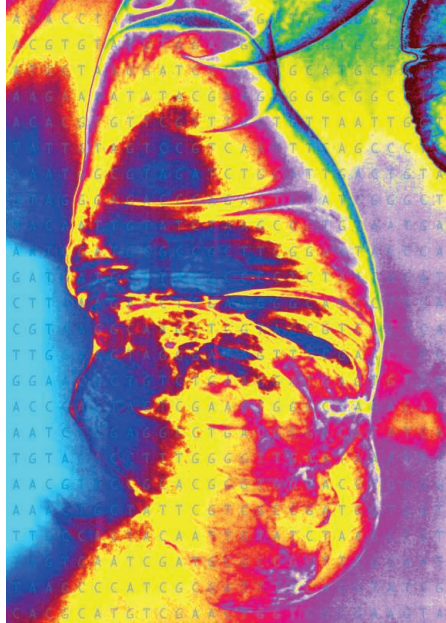
In remarks before international reporters at the start of the meeting, Press said that the scientific enterprise is "the greatest mechanism ever invented to turn human creativity into economic benefits." But countries like the United States, he warned, may not be able to capture these benefits without sustained funding for basic research.

— Becky Ham and Kathy Wren



Patient investors. William H. Press (above) and (right, front to back) panelists Anne Glover, Robert-Jan Smits, and Helga Nowotny say that countries reap economic benefits by persistently supporting basic research.





INTRODUCTION

A Medical Renaissance?

WITH THE COMPLETION OF SEQUENCING OF THE HUMAN GENOME IN 2001, MANY researchers immediately set their sights on using this information to better understand the genetics and, more recently, epigenetic effects identified during the initiation, development, and progression of cancer. Moving from the pre-genome-era identification of single gene variants associated with hereditary cancers, advances in sequencing technology have enabled the use of a whole-genome approach to examine the differences between the genomes, and epigenetic regulation, of tumor and patient DNA. This issue of *Science* examines how these advances are shaping our current understanding of cancer at the genomic level.

Unfortunately, there is no magic bullet, as no single genetic variant or epigenetic effect has been identified as a target in the fight against all cancers. In a comprehensive Review, Vogelstein *et al.* (p. 1546) distill information derived from more than 100 cancer genome sequencing projects into a series of clear principles about tumor biology and then discuss the projected clinical impact of cancer genome analysis on early detection and treatment of the disease. Kilpivaara and Aaltonen (p. 1559) call for standards for cases in which whole-genome sequencing reveals clinically relevant mutations in individual patients to bridge differences between the laboratory and the clinic. McLeod (p. 1563) reviews how best to apply pharmacogenomic information in identifying and tailoring drugs to target cancers. Finally, Suvà, Riggi, and Bernstein (p. 1567) examine how cell fates are controlled by epigenetic regulation and identify parallels between cancer and cellular differentiation.

In addition, *Science Signaling* presents related content on 26 March and 6 April, highlighting how cancer-associated alterations in the genome or proteome can result in altered signaling that contributes to tumorigenesis, metastasis, and drug resistance. Our *Science* News department profiles Elaine Mardis (p. 1540), whose expertise in developing DNA sequencing technology led her to become a pioneer in cancer genomic research, and explores how the heterogeneity of individual tumors, revealed by sequencing studies, poses treatment problems. *Science* Careers profiles Fátima Al-Shahrour, a bioinformaticist who works on interpreting the genome to help select more effective drugs for cancer patients.

Technological breakthroughs, coupled with the greatly reduced costs of sequencing, suggest that in our not-too-distant future, routine cancer treatment will not focus on the organ of origin but rather on the genomic profile of the cancer. This fundamental goal, to be able to read the complex code embedded in our bodies to identify the best therapies for each individual over the course of their treatment, portends a medical enlightenment.

— LAURA M. ZAHN AND JOHN TRAVIS

Cancer Genomics

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Science

Steering Cancer Genomics Into the Fast Lane

With her passion for DNA sequencing technology, Elaine Mardis now hopes to help cancer patients

WHEN ELAINE MARDIS COMPETED FOR HER first tae kwon do black belt, Richard Wilson, her instructor, was a little worried. The test included a side kick through a free-standing, 5-centimeter-thick pavers stone. Though a standard part of the belt test for years, the bricks were now made differently, with epoxy, and were harder to break. A dozen students failed in their first attempts that day. But not Mardis. She “hammered” it, Wilson recalls.

The concentration and drive that destroyed that brick have stood Mardis in good stead throughout her almost 30-year career in genome sequencing. Now co-director of the Genome Institute at Washington University (WU) School of Medicine in St. Louis and one of the few female leaders in the field, she jumped into DNA sequencing during its earliest days, was instrumental in helping decode the human genome faster than expected, and has since pushed the limits of the technology to learn about cancer. Today, oncologists can envision the day when they routinely sequence their patients’ tumors, but when Mardis and her colleagues broke new ground by fully deciphering the first cancer genome, most researchers considered it an impossibly difficult and expensive task. “It was a foundational piece of work,” says Paul Spellman, a cancer systems biologist at Oregon Health & Science University in Portland. “But it did not suddenly mean that everyone else could do it easily.”

Mardis and her colleagues were not content to sequence the genome of just one patient or even one cancer. They got in on the ground floor of the Cancer Genome Atlas, a massive National Institutes of Health (NIH) effort to characterize the molecular basis of 20 cancers (*Science*, 15 September 2006, p. 1553). Across many tumors, the WU group is using its technology to find genes and pathways that may work as drug targets and to see how tumors develop resistance. Her team has even helped save the life of one of their labmates after he relapsed with leukemia. By providing him with what Mardis calls the Maserati approach, a series of genomic analyses that she hopes may one day become standard for clinical care, they identified an existing drug that stopped the relapse in its tracks. Her goal now, Mardis says, is “to have an influence on patient care.”

Learning to sequence

Mardis stands out from the crowd for more than her science. She loves pink, for example, and lets people know it: Hot pink lipstick is her trademark. Today, she’s also sporting a pink winter coat and rose-colored snakeskin boots. And such playful boldness continues

into Mardis’s office, where one can’t help but notice the rows of red toy cars in all shapes and sizes. Dozens of Hot Wheels sports cars are arrayed on her desk. In front of her is a small display case with miniature Ferraris (1962 and 2002 models), reminders of “one of the most exhilarating experiences I’ve ever had,” she says, referring to her driving a friend’s Scuderia Spider in California. “I really like cars, red cars in particular.” Her current vehicle is a red Audi S5, but her dream machine is a red Porsche 911 Carrera S.

Off the road, Mardis’s machine of choice is the DNA sequencer, and the faster the better. Inspired to study science by her chemistry professor father, the Nebraska native took up biology across state lines at the University of Oklahoma (OU). For graduate school, OU biochemist Bruce Roe lured her from fruit

fly genetics, where DNA was more of an “abstract entity” to molecular biology, where “I could really work with it,” she recalls. Roe soon managed to buy one of the first commercial DNA sequencing machines, and he

set Mardis loose on making that machine, as well as a primitive robot for pipetting, work for research. “We started to think, how do we scale up these methods to produce a data production pipeline?” Mardis says.

With the beginnings of a pipeline, she deciphered 5000 hard-to-sequence DNA bases from a bacterium for her Ph.D. thesis, a remarkable feat back in 1989. By that point, however, technology development had become her passion. After graduating, she

“My love of technology hasn’t changed, but my job description has. I feel a sense of urgency with cancer genomics.”
—Elaine Mardis

Melding cancer and genomics. Timothy Ley, Elaine Mardis, and Richard Wilson (*left to right*) brought whole genome sequencing to bear on leukemia.

went to work for Bio-Rad, a California company that was developing a new polymerase, an enzyme important for DNA sequencing. There she learned all about management styles—good and bad—and how to set up production lines for biological products, knowledge that she put to good use when Wilson, who had also worked in Roe's lab, recruited her for WU's new genome center in 1993. Her job: developing instruments and techniques for high-throughput DNA sequencing.

Within months, Mardis had come up with a new strategy for isolating the DNA targeted for sequencing. At the time, a virus called M13 was used in bacteria to make many copies of the DNA to be sequenced, but then that DNA had to be separated from the bacteria's own genetic material. Mardis's approach was more efficient, less caustic, less smelly, and didn't yield as much hazardous waste as the traditional phenol-based extraction method. That was just the first of many improvements she has made in the past 20 years, during which she and her colleagues sequenced the first animal genome in 1998, *Caenorhabditis elegans*, and then made a frenzied push to complete the human genome ahead of a private company (*Science*, 16 February 2001, p. 1177). Her technology development group swelled to 2 dozen people, including electrical and mechanical engineers, as they worked to come up with ways to automate and standardize the preparation and sequencing of DNA samples. "We set aggressive goals that at the time we set them weren't possible. But failure wasn't an option, so you just rolled up your sleeves and figured out how to do it," Mardis says.

Her peers were impressed. "In a way, she served as a model for me," says Chad Nusbaum, who was involved with scaling up human DNA sequencing at the Whitehead Institute in Cambridge, Massachusetts. "We were all tackling a big challenge. She did it with more verve than most."

By 2001, a rough draft of the human genome was done. (The public and private efforts arguably tied.) As they finished some of the last details on that genome, WU's sequencers began knocking out additional ones, tackling the mouse, chicken, platypus, corn, and zebra finch genomes. Gradually, the Genome Center, which was renamed the Genome Institute 2 years ago, shifted into sequencing the DNA of more and more people. Since 2010, the center has really ramped

up its efforts in cancer, such that today, 60% of the DNA studied comes from human cancers.

Part of that shift was made possible by the cheaper and faster sequencing technology that began to appear in the mid-2000s. "Really seeing for the first time what would be possible" with these new machines was a "eureka moment," Mardis recalls.

But while sequencing technology brokers still see her as the go-to person, and she's a founder and organizer of the annual DNA sequencing conference that's become the place to announce new technologies and hobnob with industry representatives, a new passion, battling cancer, has begun to push her. "My love of technology hasn't changed, but my job description has," Mardis says. "I feel a sense of urgency with cancer genomics."

Tumor whole genomes

Amidst the toy cars on her desk is a sign of that urgency: *The Biology of Cancer*, the classic textbook by geneticist Robert Weinberg. A crash education through reading that tome, attending cancer meetings, and talking to physicians has helped her learn the language of the many oncologists now seeking her aid in harnessing genome sequencing. (Ironically, Weinberg has publicly questioned whether cancer genomics will help patients more than traditional methods of studying individual genes.)

Eight months ago, for example, after giving a talk in Italy, she was approached by a Harvard University physician who for years has collected tissue samples from lung cancer patients before and after their tumors had become resistant to drug treatments. He is now sharing those samples with Mardis, hoping that DNA sequencing will clarify the dynamics of the tumors. Her technology expertise had given her the confidence to be the first to try whole genome sequencing of tumors, and now she's involved with genome studies of seven cancers—of the breast, brain,

liver, prostate, pancreas, lung, and blood.

This run began in 2007 when she, Wilson, and Timothy Ley, a WU oncologist located a few blocks away, were in the midst of trying to use genomics to find muta-

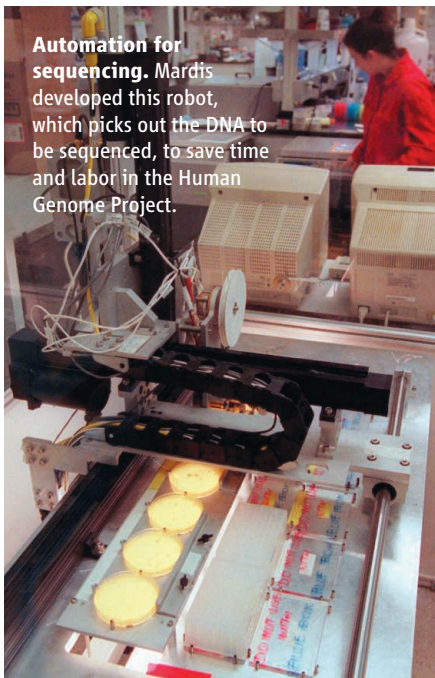
tions underlying acute myeloid leukemia (AML), which affects 13,000 people in the United States each year and kills 8800. Until then, cancer genomics consisted primarily of looking for large-scale aberrations in chromosomes, such as translocations, and studying genes that were suspect because they had already been implicated in cancer. Instead of just going after individual genes, the trio wondered, why not use next-generation sequencing technology to try to read a whole genome

from tumor cells, and for comparison, from healthy cells in the same patient. Others had sequenced the exomes, or protein-coding regions, of the DNA in tumor cells, which represent just a small percentage of the whole genome, to try to find mutations. The whole genome should provide a more complete picture, pinpointing deleted genes and changes in regulatory regions as well as in genes themselves.

They proposed the project, estimated to cost \$1 million, to NIH as part of their grant renewal, but the study section reviewing it "hated it," Mardis recalls. Unfazed, they pitched their case to a local philanthropist, Alvin J. Siteman, after whom WU's cancer center is named. The next day, he transferred stocks worth \$1 million to WU for the work, spurring a Human Genome Project-like frenzy among the researchers. They spent 16 months learning how to work with and then analyze the short pieces of DNA sequence produced by the next-generation sequencers. "It turned out to be very difficult," says Gad Getz, who is involved in cancer genomics at the Broad Institute in Cambridge. "In terms of analysis, it was a nightmare."

But the resulting paper, published in *Nature* in 2008, was a real eye-opener. The

Automation for sequencing. Mardis developed this robot, which picks out the DNA to be sequenced, to save time and labor in the Human Genome Project.



team pinpointed 10 relevant mutations, two of which were already linked to AML and eight that were new to oncologists. “The effect was that people saw that it was actually possible to sequence a whole tumor genome,” Getz says. “Very many people were excited.” Suddenly, sequencing looked like it might eventually have a place in cancer care. They “started the idea of doing personal genomics” with whole genomes for cancer patients,” Spellman says.

That first AML sample came from a woman in her mid-50s who had responded to treatment at first, then relapsed a year later and died a year after that. The group described a second AML patient’s genome in 2009 in *The New England Journal of Medicine*—the tumor cells had 12 mutations in genes and about 50 base changes in putative or known regulatory regions. Their further studies of other people with AML demonstrated that mutations in two genes, *IDH1* and *DNMT3A*, help predict a patient’s outcome. Patients have different portfolios of mutations, Mardis explains, but the hope is that by sequencing enough AML cancers, patterns will emerge that will reveal a common set of mutations, or at least pathways affected by the diversity of mutations, that can be specifically targeted for therapy.

For eight AML patients, the WU group sequenced cancer cells when each person was first diagnosed and when the patient relapsed after chemotherapy. The relapse cancer came from the same pool of bone marrow cells that had led to cancer in the first place, but those cells had undergone additional mutations, they reported in the 11 January 2012 issue of *Nature*. “We were the first to show that, genome-wide, chemotherapy had a ‘signature’ of DNA damage,” Mardis. “It confirms what was suspected for some time. By using chemotherapy, we may be setting up the patient to relapse because of acquired new mutations.”

Toward the clinic

Cancer genomics became very personal for Mardis 2 years ago. In July 2011, Lukas Wartman, an oncologist and postdoctoral fellow in Ley’s lab, who had been successfully treated for acute lymphoblastic leukemia (ALL) and had relapsed once before, relapsed again. Ley and Mardis had just begun a genome sequencing project for ALL, so they deciphered all the DNA from a sample of Wartman’s cancer cells and also analyzed the cancer’s transcriptome, that is, all the cells’ RNA, to see which genes were active. “It seemed more of an academic exercise and not something that would change

the course of my treatment,” Wartman recalls. But as aggressive chemotherapy failed, and he became very sick, the project became a race against time to find another solution.

It took about 4 weeks to get the DNA results; they were disappointing. Of the many mutations found in Wartman’s cancer cells, none were known drug targets. A few days later, however, the transcriptome study revealed excessive amounts of RNA for a gene called *FLT3*, which helps cells grow and divide.

Fortuitously, as part of an effort to streamline the analyses of cancer genomes and tran-



A way of life. The discipline and drive that Mardis brings to tae kwon do extends to her work as well.

scriptomes, Obi and Malachi Griffith, twin bioinformaticists working for Mardis, had developed a database that links gene mutations to small molecule drugs that inhibit those genes’ resulting proteins. The motivation is that using such targeted drugs would have fewer side effects than conventional chemotherapy and not cause further mutation in the tumors. In their database, the research team found a drug that could counter *FLT3*’s growth-stimulating activity; the company that owned its rights had already received Food and Drug Administration approval to treat kidney cancer with it.

The treatment worked: Once again, Wartman is in remission. The satisfaction of finding something useful for a cancer patient is “a feeling I want to have more often,” Mardis says.

Toward that end, she and her colleagues

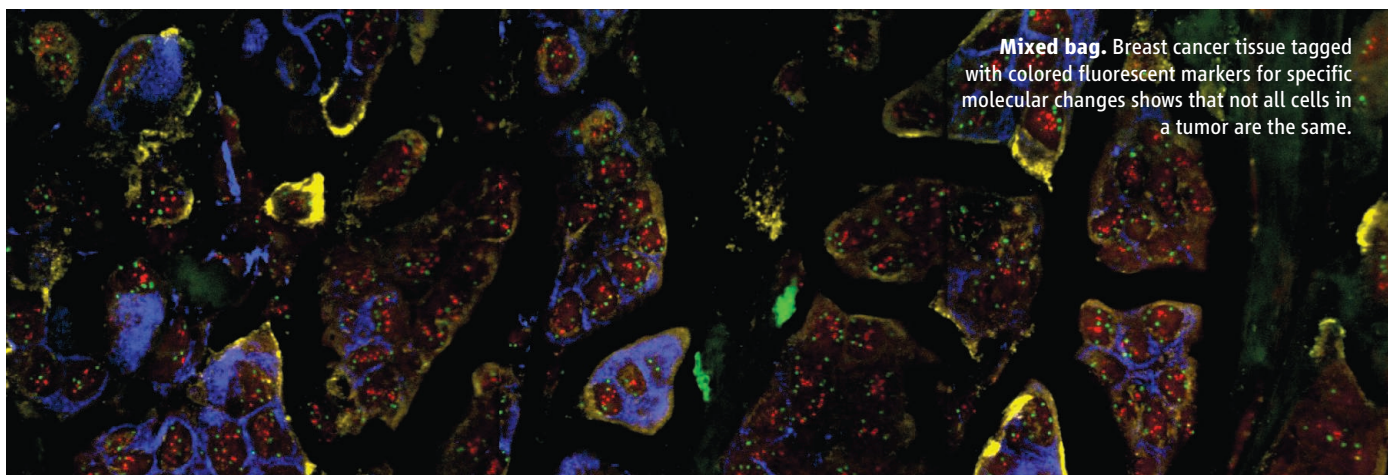
have become more focused on how to use sequencing data in the clinic. Mardis argues that for now, whole genome data need to be supplemented with transcriptome and even exome information to get the most comprehensive picture possible—the so-called Maserati approach. Matthew Ellis, a WU oncologist who has closely worked with Mardis on breast cancer genomics, would like to see the proteome—details about the proteins present in a tumor cell—included as well in a patient’s workup. “They are pioneers, and now the whole field is moving in that direction,” Getz says.

The push toward the clinic has required more technology development. Mardis’s group has been coming up with ways to deal with patient samples that are too small for easy DNA sequencing or that are difficult to process because they have been preserved in formalin and embedded in paraffin. At the same time, the team is working to shorten the time between getting a sample and generating usable results. Right now, it takes about a month; they want it to take a few days at most. They are trying out a new machine by Illumina that promises to cut the sequencing time from 10 days to 1 day.

Mardis is also exploring the uses of genomics data in other contexts. One new breast cancer project intends to use genomics to figure out if there are proteins uniquely produced by a person’s tumor that can become targets for personalized immunotherapy or vaccinations against recurrences. A childhood brain cancer effort uses genomics to get transcriptome readouts from microglia cells, supportive cells in the brain that may provide cancer-stimulating signals to brain cancer cells.

To fit all these projects into her schedule, Mardis gets up at 4:30 a.m. and is at her desk by 7, traveling 48 kilometers from a French country-style house she and her husband built in 2011. They are empty-nesters, except for two Yorkshire terriers and a German shepherd; their daughter is a college senior. Mardis has been away from home roughly 150 days a year for the past several years, giving so many talks and workshops that recently Wilson put a “No!” sign on her computer monitor to try to keep her from accepting every invitation and project that comes along. But Mardis’s new sense of urgency rarely lets her follow that advice—and cancer patients of the future may be glad for that. Roe says, “She continues to come up with new ideas, new ways of doing things and new ways of asking questions.”

—ELIZABETH PENNISI



Mixed bag. Breast cancer tissue tagged with colored fluorescent markers for specific molecular changes shows that not all cells in a tumor are the same.

The Downside of Diversity

Increasing genetic evidence that tumors contain a heterogeneous mix of cells may explain why cancer treatments often fail

AS AN ONCOLOGIST, CHARLES SWANTON TOO often has to tell his patients with advanced lung and breast cancer that their options are running out. That despite several different treatments, each somewhat successful at first, their tumors have grown back yet again, faster than ever. “It’s as though tumors have this ability to guess what you are about to do next and preempt it,” he laments.

Swanton now thinks that he has evidence explaining how they might do that. When the research team he leads at Cancer Research UK’s London Research Institute recently sequenced DNA taken from different parts of a patient’s kidney tumor, the results did not agree. While several genetic changes were shared throughout the original tumor mass and other tumors, or metastases, that sprang from it, most were present in only some parts, suggesting that tumors harbor diverse populations of cells. Some of these may be resistant to a treatment and become unleashed by it to grow, Swanton concluded: “To me, this begins to explain why drugs and therapies stop working.”

Swanton’s results have not only confirmed a hunch that he says many clinicians share, but they have also brought him a measure of unexpected scientific fame. His team’s report last March in *The New England Journal of Medicine (NEJM)* on the genetic diversity within individual kidney tumors attracted 143 citations by year’s end, more than any other original research paper in biomedicine in 2012, according to the company Thomson Reuters, which tracks the impact of scien-

tific papers. And Swanton’s e-mail inbox is overflowing with speaking invitations. “I’ve never been so busy in all my life,” says the 41-year-old who started his own lab just 5 years ago.



“To me, this begins to explain why drugs and therapies stop working.”

**—Charles Swanton,
Cancer Research UK**

Genomics studies like Swanton’s are stirring new interest in an old idea—tumors are a mosaic of different cells—by confirming with modern techniques that tumors are

not always dominated by genetically identical cells, as once thought. Instead, tumors often contain many subsets of cells that are related but genetically distinct. As a tumor evolves, identical cells apparently split off and develop new mutations or other errors, like a tree growing many branches. This means that two parts of the same tumor, as well as the metastases that form when cancer spreads, can look very different genetically.

The extent of this intratumor heterogeneity has shaken cancer biologists and clinicians. They’re rethinking major projects to tally up cancer mutations, for example, because the data so far have been based on a single sample for each tumor type. And physicians worry that the new studies suggest that the growing practice of analyzing the genetic and molecular characteristics of a single tumor biopsy to guide patient treatment may sometimes mislead. A cancer’s heterogeneity could also account for why much-heralded new drugs that target specific gene mutations may keep tumors in check for a time but almost inevitably stop working; they may merely lift a lid on a small population of drug-resistant cells and give them a chance to grow.

“What it’s leading us toward is a kind of understanding that cancer is a very slippery beast. There’s so much competition and so much evolution within a given cancer that it will be able to evade many of the treatments that we throw at it,” says cancer geneticist Peter Campbell of the Wellcome Trust Sanger Institute in Hinxton, U.K.

Some researchers say that not all is lost, however. A better handle on intratumor heterogeneity could lead to more effective treatments. “It’s daunting, but it’s exciting because we now have the tools” to understand what drives diversity in a tumor, says

Kornelia Polyak of the Dana-Farber Cancer Institute in Boston, who studies intratumor heterogeneity in breast cancer.

Clone wars

Pathologists have known since they first put tumor tissue under a microscope in the mid- to late 1800s that tumors aren't made up of uniform-looking cells, hinting at underlying biological heterogeneity. In the 1970s and early 1980s, cancer biologists showed that cells taken from different parts of a tumor varied in their ability to form tumors and spread in mice and in how the cells responded to drugs.

The notion that tumors contained a diverse range of cells fit with the clonal evolution theory of cancer, put forth in 1976 in *Science* by cancer biologist Peter Nowell, saying that cancer develops when a single cell randomly acquires a series of mutations that allow it to multiply and outcompete other, differently mutated cells, or clones, in a kind of Darwinian competition. However, Nowell assumed the less fit clones died out—which meant that one clone should make up most of the tumor mass and any other cells should be less mutated versions of that clone. That view later gained increased credibility as Johns Hopkins University researcher Bert Vogelstein and a co-worker applied it to colon cancer, describing a slow, linear accumulation of specific genetic changes that transform a normal epithelial cell into a polyp and then a growing tumor, dominated by a single clone.

Yet in the past decade, Polyak's lab and others testing tumors for cancer-related abnormalities, such as specific mutated genes, extra copies of genes, or abnormal levels of proteins, have found variation within tumors, suggesting that many distinct subpopulations of cells, known as subclones, exist. Only when DNA sequencing costs dropped and researchers could probe tumors at the depth needed to find rare mutations, however, did they begin to glimpse the full picture of genetic diversity within a single cancer.

In the last 4 years, for example, several research teams worldwide, including one led by Elaine Mardis of Washington University School of Medicine in St. Louis (see p. 1540), sequenced the genomes of breast tumors and found that a woman's metastases differed genetically from her primary tumor and appear to have evolved from subclones within that original tumor. Vogelstein's team sequenced different parts of several pancreatic tumors and found a series of genetically diverse subclones had evolved over time,

some of which had split off from the primary tumor to form the patient's metastases outside the pancreas. Several genetics studies of leukemias, including genome sequencing by Mardis's group, have shown that these cancers of the blood sometimes harbor many rare subclones.

These experiments, which suggested a branching evolution model for many cancers rather than a linear process (see diagram), usually tested blood or ground-up biopsy



“There will be a lot of investment in this area and maybe trials, but I think it will take some time to figure out what really helps [overcome tumor heterogeneity].”

**—Kornelia Polyak,
Dana-Farber Cancer Institute**

samples, so the results reflect a mixture of cells. In another approach to studying heterogeneity, Nicholas Navin and others in the lab of geneticist Michael Wigler at Cold Spring Harbor Laboratory in New York have sequenced the DNA of single cells taken from breast cancer biopsies. They found distinct populations of cells, including some that had acquired new mutations in a rapid burst, like Darwin's “punctuated evolution,” rather than developed them in a gradual manner as had been believed, says Navin, who is now at the University of Texas MD Anderson Cancer Center in Houston.

Perhaps the most dramatic study yet of genetic heterogeneity within a solid tumor is the one led by Swanton's lab. In addition to conducting other molecular tests, the

researchers methodically sequenced the protein-coding DNA of samples snipped from various locations on a large kidney tumor and from the patient's metastases. They reported that of the 128 mutations identified in one patient, only about one-third were shared by all the samples. The level of heterogeneity was similar for three more patients' tumors. Some parts of a tumor had different mutations in the same cancer genes, showing that subgroups of cells had evolved a defect in the same gene independently.

The good news, Swanton says, is that some growth-spurring “driver” genetic changes present early in the evolution of the patient's tumor persisted as it grew and spread, which means that a drug targeting those abnormal genes should work on most of the cancerous cells. However, his and other studies also suggest that even if a drug works at first on metastatic cancer, it probably won't work over the long term because tumors harbor rare clones with additional genetic changes that may enable them to resist the drug, Swanton says.

The resistance may also come from heterogeneity caused by sources other than mutated genes, some researchers say. They are finding evidence that chemical modifications to the DNA or other molecular fluctuations within the cell that turn genes off or on may allow some tumor cells to overcome cancer drugs or chemotherapy. “It certainly makes it more complicated, that's for sure,” says John Dick of the University of Toronto in Canada. (He led a study with Shibata, published in the 1 February issue of *Science*, suggesting that genetically similar cells in colon tumors can differ in their resistance to drugs [p. 543].)

Tumor heterogeneity, whether from DNA mutations or other changes in the cells, is a persuasive explanation of why treatments fail, Polyak says. She goes so far as to suggest that it explains resistance better than the “cancer stem cell” theory, which posits that resistance occurs when a rare, drug-resistant cell expands to dominate the tumor mass.

Provocative questions

To some who study the evolution of cancer, the accumulating evidence of genetic tumor heterogeneity is welcome but unsurprising—and they worry that it will do little for treatment. “It's all descriptive,” says Darryl Shibata of the University of Southern California in Los Angeles. “The critical question is, is heterogeneity of any scientific and practical value? Its actual meaning is unclear.”

That hasn't stopped leaders of the Cancer Genome Atlas, a large project partly funded by the U.S. National Cancer Institute (NCI) in Bethesda, Maryland, from taking steps to address tumor heterogeneity. One follow-up project that they are considering is to go back and sequence leftover samples from primary tumors and, when available, metastases, to explore how the cancers evolved, says NCI's Stephen Chanock. The Sanger Institute's Campbell expects that other cancer genome projects in the United Kingdom and elsewhere will begin sequencing samples of both primary tumors and their metastases. Some researchers even argue that these projects may have missed the most important, deadliest mutations by focusing on only single biopsies. "It's very embarrassing because they should have done this at the very beginning," Shibata says.

Campbell sees at least one clinical change coming out of the work on tumor heterogeneity: Physicians will need to sequence primary and metastatic tumors, when feasible, if they're tailoring therapies to an individual. They may also need to biopsy tumors throughout the course of a patient's disease to follow the cancer's evolution, he and others say.

A major unresolved issue swirling around tumor heterogeneity is whether patients with more genetically diverse tumors have a poorer prognosis, Swanton says. They do, according to some early evidence from a team at the Dana-Farber Cancer Institute and the Broad Institute.

The researchers reported in *Cell* in February that among people with chronic lymphocytic leukemia who received chemotherapy, those whose original leukemia harbored subclones with one or more cancer-driver genes needed retreatment more often or died sooner than people without this diversity. Campbell says his group, too, is finding that in patients with a preleukemia condition called myelodysplasia, those with an aggressive subclone in their tumor before treatment develop leukemia and die sooner.

Some ambitious researchers would like to be more precise about heterogeneity in a person's cancer, possibly ranking it on a

graded scale, Navin says, to improve treatments. For instance, Carlo Maley, director of the Center for Evolution and Cancer at the University of California, San Francisco, who has modeled the progression of Barrett's esophagus, a precancerous condition in which cells in the lower esophagus acquire an abnormal appearance, showed several years ago that patients with more genetically diverse premalignant cells as measured on a

drugs in such cases will drastically reduce the odds that the cancer will develop resistance.

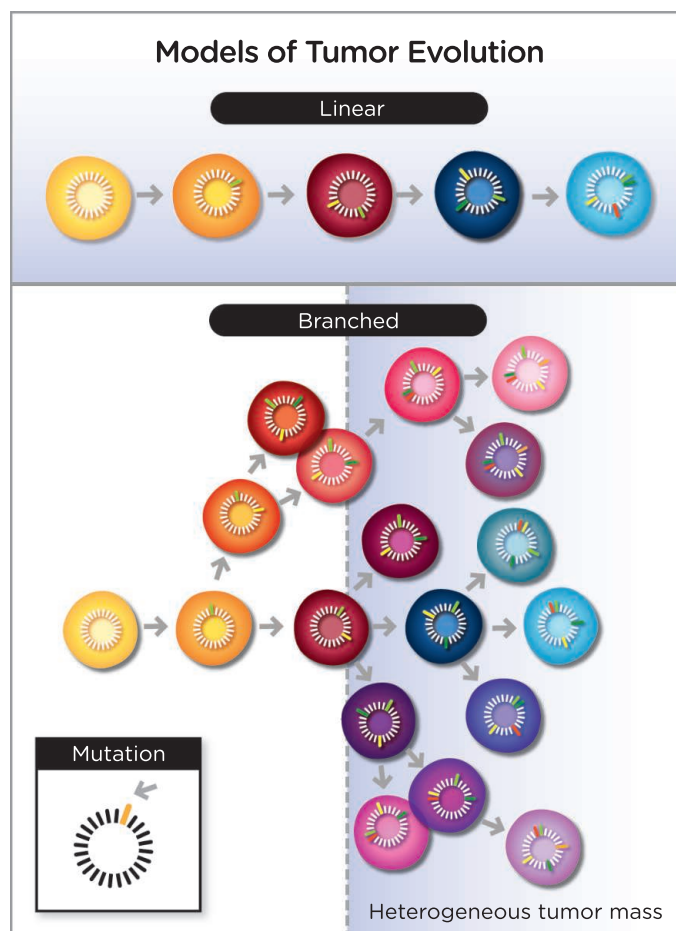
Swanton wants to defeat heterogeneity by preventing it. His group recently reported that in colon cancer, cells are often missing some genes that make sure DNA is copied correctly during cell division, which can result in chromosomal abnormalities. Gene-based drugs that restore proper copying, or other processes that keep a cell's DNA error-free, might one day limit a tumor's diversity, making it less likely to develop resistance, he suggests.

A more radical approach is to forget about wiping out all the cells in a tumor and instead just keep it from growing. Robert Gatenby, a radiologist at the Moffitt Cancer Center in Tampa, Florida, argues that the drug-resistant cells in a tumor are kept in check by the cells that dominate the tumor mass, and it's best to let both types of cells coexist. Gatenby has reported that mice implanted with aggressive ovarian cancer tumors live at least twice as long, or 4 months, if they are given doses of chemotherapy so low that the drugs don't wipe out tumors but instead keep them stable in size. "We need to be respectful of evolution and its effects on therapy and try to use it instead of letting it defeat us," says Gatenby, who calls his strategy "adaptive therapy."

When he submitted a research proposal to expand this work in 2009, he received "the worst reviews I ever got," Gatenby says. But last fall, he won a \$2.1 million grant from NCI's Provocative Questions program, which encourages researchers to test out-of-the-box ideas. Gatenby soon hopes to launch a small clinical trial in which prostate cancer patients will receive smaller than normal doses of antiandrogen therapy, which the researchers will regularly tweak by monitoring tumors with imaging and using mathematical models to predict their growth.

Polyak thinks such strategies for overcoming intratumor heterogeneity are worth pursuing. "There will be a lot of investment in this area and maybe trials, but I think it will take some time to figure out what really helps," Polyak says.

—JOCELYN KAISER



Two views. Tumors were long thought to evolve in a linear fashion as a single cell acquired growth-spurring mutations and dominated the final mass. But new studies indicate that in many tumors, cells branch off and form a diverse tumor with cells that may evade treatments.

numerical scale are more likely to develop full-blown cancer.

Some remain optimistic that the diversity of tumors doesn't mean that targeted drugs are doomed to fail. Vogelstein suggests that because most patients' primary tumors are removed with surgery, only the heterogeneity in the metastatic tumors matters (see p. 1546). Some of those patients have metastases with mutations in two different but fundamental growth pathways that can each be targeted with a drug; Vogelstein argues that combining

REVIEW

Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shibin Zhou, Luis A. Diaz Jr., Kenneth W. Kinzler*

Over the past decade, comprehensive sequencing efforts have revealed the genomic landscapes of common forms of human cancer. For most cancer types, this landscape consists of a small number of “mountains” (genes altered in a high percentage of tumors) and a much larger number of “hills” (genes altered infrequently). To date, these studies have revealed ~140 genes that, when altered by intragenic mutations, can promote or “drive” tumorigenesis. A typical tumor contains two to eight of these “driver gene” mutations; the remaining mutations are passengers that confer no selective growth advantage. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance. A better understanding of these pathways is one of the most pressing needs in basic cancer research. Even now, however, our knowledge of cancer genomes is sufficient to guide the development of more effective approaches for reducing cancer morbidity and mortality.

Ten years ago, the idea that all of the genes altered in cancer could be identified at base-pair resolution would have seemed like science fiction. Today, such genome-wide analysis, through sequencing of the exome (see Box 1, Glossary, for definitions of terms used in this Review) or of the whole genome, is routine.

The prototypical exomic studies of cancer evaluated ~20 tumors at a cost of >\$100,000 per case (1–3). Today, the cost of this sequencing has been reduced 100-fold, and studies reporting the sequencing of more than 100 tumors of a given type are the norm (table S1A). Although vast amounts of data can now be readily obtained, deciphering this information in meaningful terms is still challenging. Here, we review what has been learned about cancer genomes from these sequencing studies—and, more importantly, what this information has taught us about cancer biology and future cancer management strategies.

How Many Genes Are Subtly Mutated in a Typical Human Cancer?

In common solid tumors such as those derived from the colon, breast, brain, or pancreas, an average of 33 to 66 genes display subtle somatic mutations that would be expected to alter their protein products (Fig. 1A). About 95% of these mutations are single-base substitutions (such as C>G), whereas the remainder are deletions or insertions of one or a few bases (such as CTT>CT) (table S1B). Of the base substitutions, 90.7% result in missense changes, 7.6% result in nonsense changes, and 1.7% result in alterations of splice sites or untranslated regions immediately adjacent to the start and stop codons (table S1B).

Certain tumor types display many more or many fewer mutations than average (Fig. 1B). Notable among these outliers are melanomas and lung tumors, which contain ~200 nonsynonymous mutations per tumor (table S1C). These larger numbers reflect the involvement of potent mutagens (ultraviolet light and cigarette smoke, respectively) in the pathogenesis of these tumor types. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers (4). Tumors with defects in DNA repair form another group of outliers (5). For example, tumors with mismatch repair defects can harbor thousands of mutations (Fig. 1B), even more than lung tumors or melanomas. Recent studies have shown that high numbers of mutations are also found in tumors with genetic alterations of the proofreading domain of DNA polymerases POLE or POLD1 (6, 7). At the other end of the spectrum, pediatric tumors and leukemias harbor far fewer point mutations: on average, 9.6 per tumor (table S1C). The basis for this observation is considered below.

Mutation Timing

When do these mutations occur? Tumors evolve from benign to malignant lesions by acquiring a series of mutations over time, a process that has been particularly well studied in colorectal tumors (8, 9). The first, or “gatekeeping,” mutation provides a selective growth advantage to a normal epithelial cell, allowing it to outgrow the cells that surround it and become a microscopic clone (Fig. 2). Gatekeeping mutations in the colon most often occur in the *APC* gene (10). The small adenoma that results from this mutation grows slowly, but a second mutation in another gene, such as *KRAS*, unleashes a second round of clonal growth that allows an expansion of cell number (9). The cells with only the *APC* mutation may persist, but their cell numbers are small compared with the cells that

have mutations in both genes. This process of mutation followed by clonal expansion continues, with mutations in genes such as *PIK3CA*, *SMAD4*, and *TP53*, eventually generating a malignant tumor that can invade through the underlying basement membrane and metastasize to lymph nodes and distant organs such as the liver (11). The mutations that confer a selective growth advantage to the tumor cell are called “driver” mutations. It has been estimated (12) that each driver mutation provides only a small selective growth advantage to the cell, on the order of a 0.4% increase in the difference between cell birth and cell death. Over many years, however, this slight increase, compounded once or twice per week, can result in a large mass, containing billions of cells.

The number of mutations in certain tumors of self-renewing tissues is directly correlated with age (13). When evaluated through linear regression, this correlation implies that more than half of the somatic mutations identified in these tumors occur during the preneoplastic phase; that is, during the growth of normal cells that continuously replenish gastrointestinal and genitourinary epithelium and other tissues. All of these pre-neoplastic mutations are “passenger” mutations that have no effect on the neoplastic process. This result explains why a colorectal tumor in a 90-year-old patient has nearly twice as many mutations as a morphologically identical colorectal tumor in a 45-year-old patient. This finding also partly explains why advanced brain tumors (glioblastomas) and pancreatic cancers (pancreatic ductal adenocarcinomas) have fewer mutations than colorectal tumors; glial cells of the brain and epithelial cells of the pancreatic ducts do not replicate, unlike the epithelial cells lining the crypts of the colon. Therefore, the gatekeeping mutation in a pancreatic or brain cancer is predicted to occur in a precursor cell that contains many fewer mutations than are present in a colorectal precursor cell. This line of reasoning also helps to explain why pediatric cancers have fewer mutations than adult tumors. Pediatric cancers often occur in non-self-renewing tissues, and those that arise in renewing tissues (such as leukemias) originate from precursor cells that have not renewed themselves as often as in adults. In addition, pediatric tumors, as well as adult leukemias and lymphomas, may require fewer rounds of clonal expansion than adult solid tumors (8, 14). Genome sequencing studies of leukemia patients support the idea that mutations occur as random events in normal precursor cells before these cells acquire an initiating mutation (15).

When during tumorigenesis do the remaining somatic mutations occur? Because mutations in tumors occur at predictable and calculable rates (see below), the number of somatic mutations in tumors provides a clock, much like the clock used in evolutionary biology to determine species

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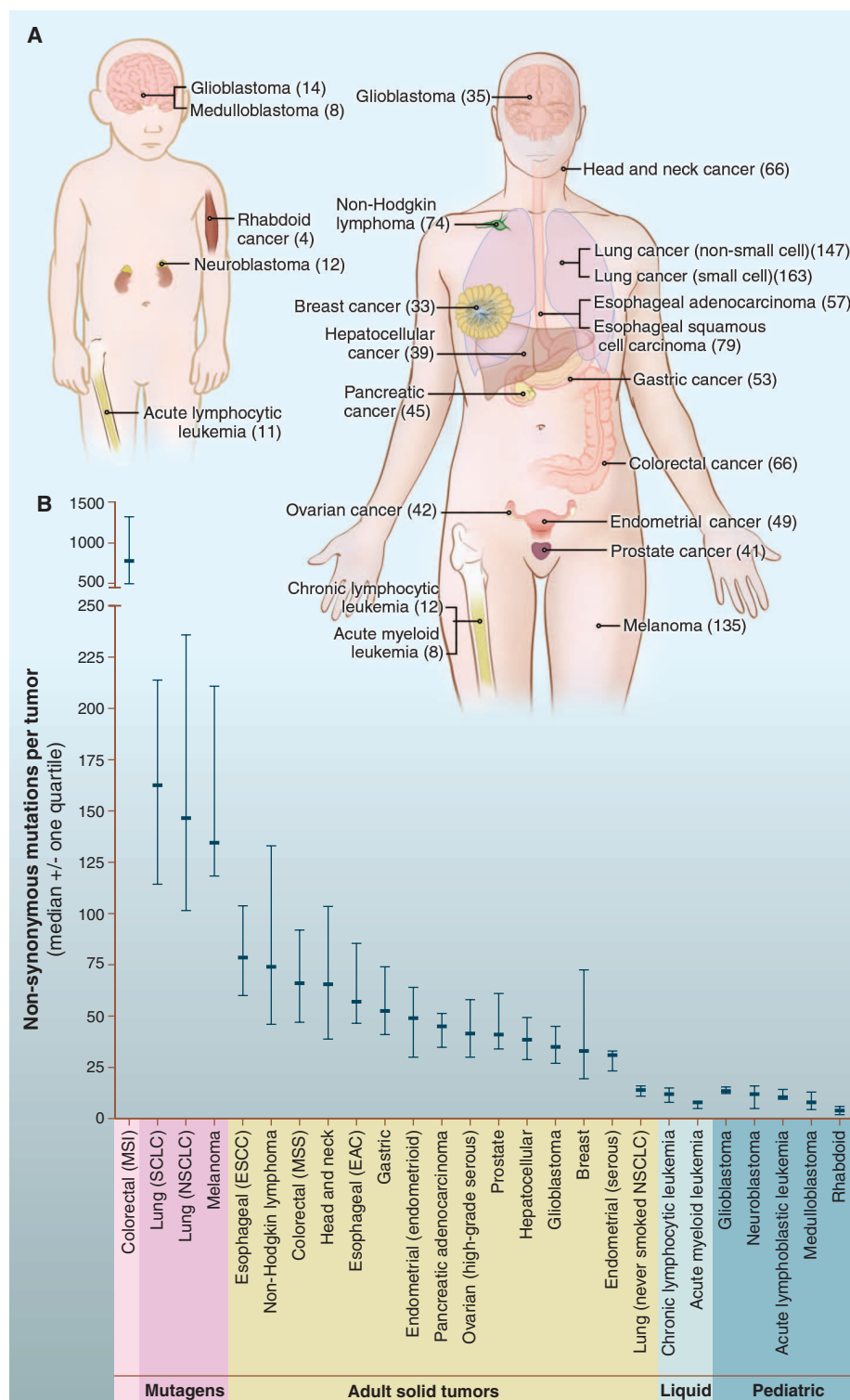


Fig. 1. Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies. (A) The genomes of a diverse group of adult (right) and pediatric (left) cancers have been analyzed. Numbers in parentheses indicate the median number of nonsynonymous mutations per tumor. (B) The median number of nonsynonymous mutations per tumor in a variety of tumor types. Horizontal bars indicate the 25 and 75% quartiles. MSI, microsatellite instability; SCLC, small cell lung cancers; NSCLC, non-small cell lung cancers; ESCC, esophageal squamous cell carcinomas; MSS, microsatellite stable; EAC, esophageal adenocarcinomas. The published data on which this figure is based are provided in table S1C.

divergence time. The number of mutations has been measured in tumors representing progressive stages of colorectal and pancreatic cancers (11, 16). Applying the evolutionary clock model to these data leads to two unambiguous conclusions: First, it takes decades to develop a full-blown, metastatic cancer. Second, virtually all of the mutations in metastatic lesions were already present in a large number of cells in the primary tumors.

The timing of mutations is relevant to our understanding of metastasis, which is responsible for the death of most patients with cancer. The primary tumor can be surgically removed, but the residual metastatic lesions—often undetectable and widespread—remain and eventually enlarge, compromising the function of the lungs, liver, or other organs. From a genetics perspective, it would seem that there must be mutations that convert a primary cancer to a metastatic one, just as there are mutations that convert a normal cell to a benign tumor, or a benign tumor to a malignant one (Fig. 2). Despite intensive effort, however, consistent genetic alterations that distinguish cancers that metastasize from cancers that have not yet metastasized remain to be identified.

One potential explanation invokes mutations or epigenetic changes that are difficult to identify with current technologies (see section on “dark matter” below). Another explanation is that metastatic lesions have not yet been studied in sufficient detail to identify these genetic alterations, particularly if the mutations are heterogeneous in nature. But another possible explanation is that there are no metastasis genes. A malignant primary tumor can take many years to metastasize, but this process is, in principle, explicable by stochastic processes alone (17, 18). Advanced tumors release millions of cells into the circulation each day, but these cells have short half-lives, and only a minuscule fraction establish metastatic lesions (19). Conceivably, these circulating cells may, in a nondeterministic manner, infrequently and randomly lodge in a capillary bed in an organ that provides a favorable microenvironment for growth. The bigger the primary tumor mass, the more likely that this process will occur. In this scenario, the continual evolution of the primary tumor would reflect local selective advantages rather than future selective advantages. The idea that growth at metastatic sites is not dependent on additional genetic alterations is also supported by recent results showing that even normal cells, when placed in suitable environments such as lymph nodes, can grow into organoids, complete with a functioning vasculature (20).

Other Types of Genetic Alterations in Tumors

Though the rate of point mutations in tumors is similar to that of normal cells, the rate of chromosomal changes in cancer is elevated (21). Therefore, most solid tumors display widespread changes in chromosome number (aneuploidy), as well as deletions, inversions, translocations,

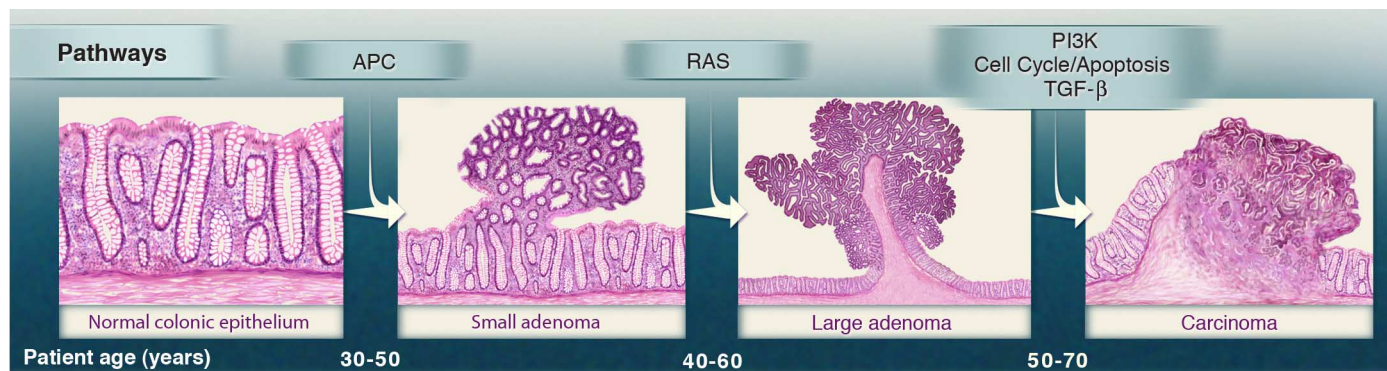


Fig. 2. Genetic alterations and the progression of colorectal cancer. The major signaling pathways that drive tumorigenesis are shown at the transitions between each tumor stage. One of several driver genes that encode compo-

nents of these pathways can be altered in any individual tumor. Patient age indicates the time intervals during which the driver genes are usually mutated. Note that this model may not apply to all tumor types. TGF-β, transforming growth factor-β.

and other genetic abnormalities. When a large part of a chromosome is duplicated or deleted, it is difficult to identify the specific “target” gene(s) on the chromosome whose gain or loss confers a growth advantage to the tumor cell. Target genes are more easily identified in the case of chromosome translocations, homozygous deletions, and gene amplifications. Translocations generally fuse two genes to create an oncogene (such as *BCR-ABL* in chronic myelogenous leukemia) but, in a small number of cases, can inactivate a tumor suppressor gene by truncating it or separating it from its promoter. Homozygous deletions often involve just one or a few genes, and the target is always a tumor suppressor gene. Amplifications contain an oncogene whose protein product is abnormally active simply because the tumor cell contains 10 to 100 copies of the gene per cell, compared with the two copies present in normal cells.

Most solid tumors have dozens of translocations; however, as with point mutations, the majority of translocations appear to be passengers rather than drivers. The breakpoints of the translocations are often in “gene deserts” devoid of known genes, and many of the translocations and homozygous deletions are adjacent to fragile sites that are prone to breakage. Cancer cells can, perhaps, survive such chromosome breaks more easily than normal cells because they contain mutations that incapacitate genes like *TP53*, which would normally respond to DNA damage by triggering cell death. Studies to date indicate that there are roughly 10 times fewer genes affected by chromosomal changes than by point mutations. Figure 3 shows the types and distribution of genetic alterations that affect protein-coding genes in five representative tumor types. Protein-coding genes account for only ~1.5% of the total genome, and the number of alterations in noncoding regions is proportionately higher than the number affecting coding regions. The vast majority of the alterations in noncoding regions are presumably passengers. These noncoding

mutations, as well as the numerous epigenetic changes found in cancers, will be discussed later.

Drivers Versus Passenger Mutations

Though it is easy to define a “driver gene mutation” in physiologic terms (as one conferring a selective growth advantage), it is more difficult to identify which somatic mutations are drivers and which are passengers. Moreover, it is important to point out that there is a fundamental difference between a driver gene and a driver gene mutation. A driver gene is one that contains driver gene mutations. But driver genes may also contain passenger gene mutations. For example, *APC* is a large driver gene, but only

those mutations that truncate the encoded protein within its N-terminal 1600 amino acids are driver gene mutations. Missense mutations throughout the gene, as well as protein-truncating mutations in the C-terminal 1200 amino acids, are passenger gene mutations.

Numerous statistical methods to identify driver genes have been described. Some are based on the frequency of mutations in an individual gene compared with the mutation frequency of other genes in the same or related tumors after correction for sequence context and gene size (22, 23). Other methods are based on the predicted effects of mutation on the encoded protein, as inferred from biophysical studies (24–26). All of these

methods are useful for prioritizing genes that are most likely to promote a selective growth advantage when mutated. When the number of mutations in a gene is very high, as with *TP53* or *KRAS*, any reasonable statistic will indicate that the gene is extremely likely to be a driver gene. These highly mutated genes have been termed “mountains” (1). Unfortunately, however, genes with more than one, but still relatively few mutations (so called “hills”) numerically dominate cancer genome landscapes (1). In these cases, methods based on mutation frequency and context alone cannot reliably indicate which genes are drivers, because the background rates of mutation vary so much among different patients and regions of the genome. Recent studies of normal cells have indicated that the rate of mutation varies by more than 100-fold within the genome (27). In tumor cells, this variation can be higher and may affect whole

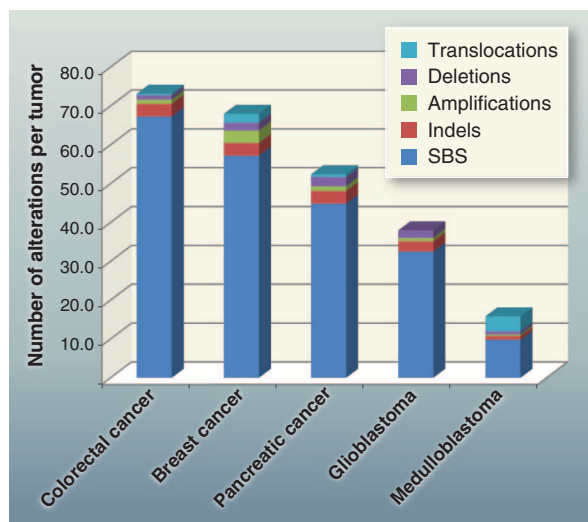


Fig. 3. Total alterations affecting protein-coding genes in selected tumors. Average number and types of genomic alterations per tumor, including single-base substitutions (SBS), small insertions and deletions (indels), amplifications, and homozygous deletions, as determined by genome-wide sequencing studies. For colorectal, breast, and pancreatic ductal cancer, and medulloblastomas, translocations are also included. The published data on which this figure is based are provided in table S1D.

Box 1. Glossary

Adenoma: A benign tumor composed of epithelial cells.

Alternative lengthening of telomeres (ALT): A process of maintaining telomeres independent of telomerase, the enzyme normally responsible for telomere replication.

Amplification: A genetic alteration producing a large number of copies of a small segment (less than a few megabases) of the genome.

Angiogenesis: the process of forming vascular conduits, including veins, arteries, and lymphatics.

Benign tumor: An abnormal proliferation of cells driven by at least one mutation in an oncogene or tumor suppressor gene. These cells are not invasive (i.e., they cannot penetrate the basement membrane lining them), which distinguishes them from malignant cells.

Carcinoma: A type of malignant tumor composed of epithelial cells.

Clonal mutation: A mutation that exists in the vast majority of the neoplastic cells within a tumor.

Driver gene mutation (driver): A mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs.

Driver gene: A gene that contains driver gene mutations (Mut-Driver gene) or is expressed aberrantly in a fashion that confers a selective growth advantage (Epi-Driver gene).

Epi-driver gene: A gene that is expressed aberrantly in cancers in a fashion that confers a selective growth advantage.

Epigenetic: Changes in gene expression or cellular phenotype caused by mechanisms other than changes in the DNA sequence.

Exome: The collection of exons in the human genome. Exome sequencing generally refers to the collection of exons that encode proteins.

Gatekeeper: A gene that, when mutated, initiates tumorigenesis. Examples include *RB*, mutations of which initiate retinoblastomas, and *VHL*, whose mutations initiate renal cell carcinomas.

Germline genome: An individual's genome, as inherited from their parents.

Germline variants: Variations in sequences observed in different individuals. Two randomly chosen individuals differ by ~20,000 genetic variations distributed throughout the exome.

Human leukocyte antigen (HLA): A protein encoded by genes that determine an individual's capacity to respond to specific antigens or reject transplants from other individuals.

Homozygous deletion: Deletion of both copies of a gene segment (the one inherited from the mother, as well as that inherited from the father).

Indel: A mutation due to small insertion or deletion of one or a few nucleotides.

Karyotype: Display of the chromosomes of a cell on a microscopic slide, used to evaluate changes in chromosome number as well as structural alterations of chromosomes.

Kinase: A protein that catalyzes the addition of phosphate groups to other molecules, such as proteins or lipids. These proteins are essential to nearly all signal transduction pathways.

Liquid tumors: Tumors composed of hematopoietic (blood) cells, such as leukemias. Though lymphomas generally form solid masses in lymph nodes, they are often classified as liquid tumors because of their derivation from hematopoietic cells and ability to travel through lymphatics.

Malignant tumor: An abnormal proliferation of cells driven by mutations in oncogenes or tumor suppressor genes that has already invaded their surrounding stroma. It is impossible to distinguish an isolated benign tumor cell from an isolated malignant tumor cell. This distinction can be made only through examination of tissue architecture.

Metastatic tumor: A malignant tumor that has migrated away from its primary site, such as to draining lymph nodes or another organ.

Methylation: Covalent addition of a methyl group to a protein, DNA, or other molecule.

Missense mutation: A single-nucleotide substitution (e.g., C to T) that results in an amino acid substitution (e.g., histidine to arginine).

Mut-driver gene: A gene that contains driver gene mutations.

Nonsense mutation: A single-nucleotide substitution (e.g., C to T) that results in the production of a stop codon.

Nonsynonymous mutation: A mutation that alters the encoded amino acid sequence of a protein. These include missense, nonsense, splice site, translation start, translation stop, and indel mutations.

Oncogene: A gene that, when activated by mutation, increases the selective growth advantage of the cell in which it resides.

Passenger mutation (passenger): A mutation that has no direct or indirect effect on the selective growth advantage of the cell in which it occurred.

Primary tumor: The original tumor at the site where tumor growth was initiated. This can be defined for solid tumors, but not for liquid tumors.

Promoter: A region within or near the gene that helps regulate its expression.

Rearrangement: A mutation that juxtaposes nucleotides that are normally separated, such as those on two different chromosomes.

Selective growth advantage (*s*): The difference between birth and death in a cell population. In normal adult cells in the absence of injury, $s = 0.000000$.

Self-renewing tissues: Tissues whose cells normally repopulate themselves, such as those lining the gastrointestinal or urogenital tracts, as well as blood cells.

Single-base substitution (SBS): A single-nucleotide substitution (e.g., C to T) relative to a reference sequence or, in the case of somatic mutations, relative to the germline genome of the person with a tumor.

Solid tumors: Tumors that form discrete masses, such as carcinomas or sarcomas.

Somatic mutations: Mutations that occur in any non-germ cell of the body after conception, such as those that initiate tumorigenesis.

Splice sites: Small regions of genes that are juxtaposed to the exons and direct exon splicing.

Stem cell: An immortal cell that can repopulate a particular cell type.

Subclonal mutation: A mutation that exists in only a subset of the neoplastic cells within a tumor.

Translocation: A specific type of rearrangement where regions from two nonhomologous chromosomes are joined.

Tumor suppressor gene: A gene that, when inactivated by mutation, increases the selective growth advantage of the cell in which it resides.

Untranslated regions: Regions within the exons at the 5' and 3' ends of the gene that do not encode amino acids.

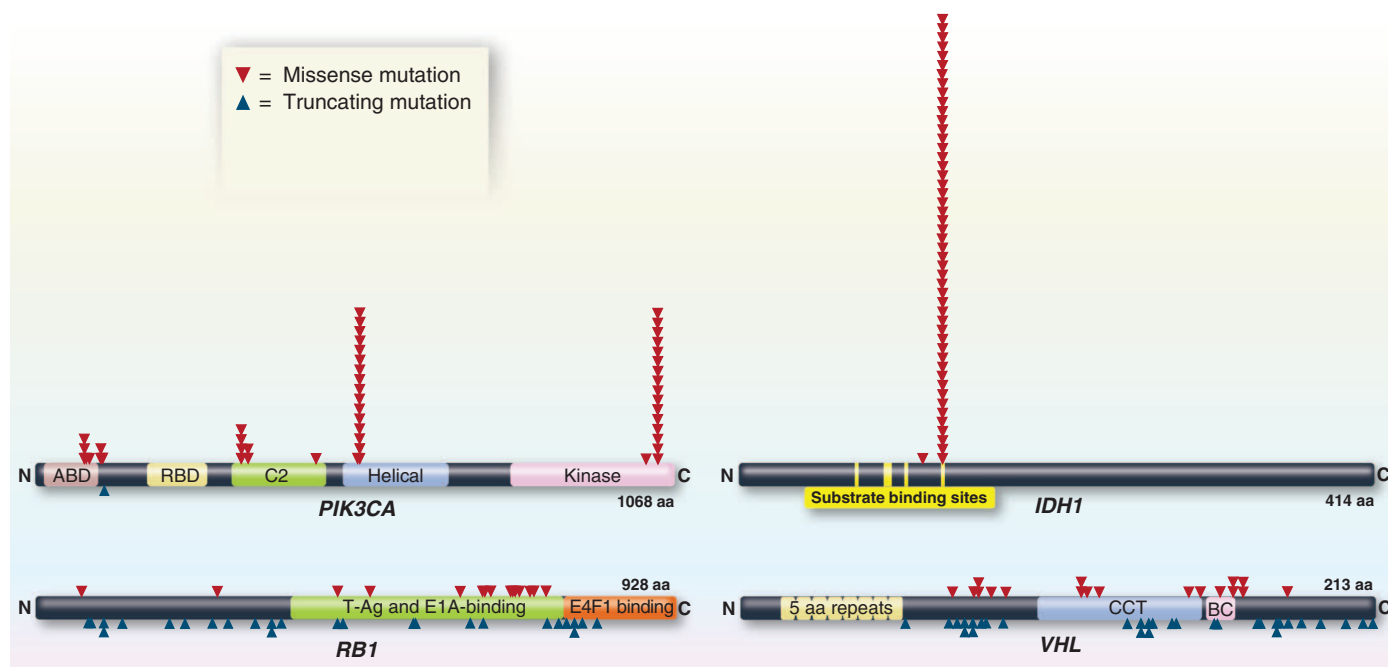


Fig. 4. Distribution of mutations in two oncogenes (*PIK3CA* and *IDH1*) and two tumor suppressor genes (*RB1* and *VHL*). The distribution of missense mutations (red arrowheads) and truncating mutations (blue arrowheads) in representative oncogenes and tumor suppressor genes are shown. The data were

collected from genome-wide studies annotated in the COSMIC database (release version 61). For *PIK3CA* and *IDH1*, mutations obtained from the COSMIC database were randomized by the Excel RAND function, and the first 50 are shown. For *RB1* and *VHL*, all mutations recorded in COSMIC are plotted. aa, amino acids.

regions of the genome in an apparently random fashion (28). Thus, at best, methods based on mutation frequency can only prioritize genes for further analysis but cannot unambiguously identify driver genes that are mutated at relatively low frequencies.

Further complicating matters, there are two distinct meanings of the term “driver gene” that are used in the cancer literature. The driver-versus-passenger concept was originally used to distinguish mutations that caused a selective growth advantage from those that did not (29). According to this definition, a gene that does not harbor driver gene mutations cannot be a driver gene. But many genes that contain few or no driver gene mutations have been labeled driver genes in the literature. These include genes that are overexpressed, underexpressed, or epigenetically altered in tumors, or those that enhance or inhibit some aspect of tumorigenicity when their expression is experimentally manipulated. Though a subset of these genes may indeed play an important role in the neoplastic process, it is confusing to lump them all together as driver genes.

To reconcile the two connotations of driver genes, we suggest that genes suspected of increasing the selective growth advantage of tumor cells be categorized as either “Mut-driver genes” or “Epi-driver genes.” Mut-driver genes contain a sufficient number or type of driver gene mutations to unambiguously distinguish them from other genes. Epi-driver genes are expressed aber-

rantly in tumors but not frequently mutated; they are altered through changes in DNA methylation or chromatin modification that persist as the tumor cell divides.

A Ratiometric Method to Identify and Classify Mut-Driver Genes

If mutation frequency, corrected for mutation context, gene length, and other parameters, cannot reliably identify modestly mutated driver genes, what can? In our experience, the best way to identify Mut-driver genes is through their pattern of mutation rather than through their mutation frequency. The patterns of mutations in well-studied oncogenes and tumor suppressor genes are highly characteristic and nonrandom. Oncogenes are recurrently mutated at the same amino acid positions, whereas tumor suppressor genes are mutated through protein-truncating alterations throughout their length (Fig. 4 and table S2A).

On the basis of these mutation patterns rather than frequencies, we can determine which of the 18,306 mutated genes containing a total of 404,863 subtle mutations that have been recorded in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (30) are Mut-driver genes and whether they are likely to function as oncogenes or tumor suppressor genes. To be classified as an oncogene, we simply require that >20% of the recorded mutations in the gene are at recurrent positions and are missense (see legend to table S2A). To be classified as a tumor suppress-

or gene, we analogously require that >20% of the recorded mutations in the gene are inactivating. This “20/20 rule” is lenient in that all well-documented cancer genes far surpass these criteria (table S2A).

The following examples illustrate the value of the 20/20 rule. When *IDH1* mutations were first identified in brain tumors, their role in tumorigenesis was unknown (2, 31). Initial functional studies suggested that *IDH1* was a tumor suppressor gene and that mutations inactivated this gene (32). However, nearly all of the mutations in *IDH1* were at the identical amino acid, codon 132 (Fig. 4). As assessed by the 20/20 rule, this distribution unambiguously indicated that *IDH1* was an oncogene rather than a tumor suppressor gene, and this conclusion was eventually supported by biochemical experiments (33, 34). Another example is provided by mutations in *NOTCH1*. In this case, some functional studies suggested that *NOTCH1* was an oncogene, whereas others suggested it was a tumor suppressor gene (35, 36). The situation could be clarified through the application of the 20/20 rule to *NOTCH1* mutations in cancers. In “liquid tumors” such as lymphomas and leukemias, the mutations were often recurrent and did not truncate the predicted protein (37). In squamous cell carcinomas, the mutations were not recurrent and were usually inactivating (38–40). Thus, the genetic data clearly indicated that *NOTCH1* functions differently in different tumor types. The idea that the same gene can function

in completely opposite ways in different cell types is important for understanding cell signaling pathways.

How Many Mut-Driver Genes Exist?

Though all 20,000 protein-coding genes have been evaluated in the genome-wide sequencing studies of 3284 tumors, with a total of 294,881 mutations reported, only 125 Mut-driver genes, as defined by the 20/20 rule, have been discovered to date (table S2A). Of these, 71 are tumor suppressor genes and 54 are oncogenes. An important but relatively small fraction (29%) of these genes was discovered to be mutated through unbiased genome-wide sequencing; most of these genes had already been identified by previous, more directed investigations.

How many more Mut-driver genes are yet to be discovered? We believe that a plateau is being reached, because the same Mut-driver genes keep being “rediscovered” in different tumor types. For example, *MLL2* and *MLL3* mutations were originally discovered in medulloblastomas (41) and were subsequently discovered to be mutated in non-Hodgkin lymphomas, prostate cancers, breast cancers, and other tumor types (42–45). Similarly, *ARID1A* mutations were first discovered to be mutated in clear-cell ovarian cancers (46, 47) and were subsequently shown to be mutated in tumors of several other organs, including those of the stomach and liver (48–50). In recent studies of several types of lung cancer (4, 51, 52), nearly all genes found to be mutated at significant

frequencies had already been identified in tumors of other organs. In other words, the number of frequently altered Mut-driver genes (mountains) is nearing saturation. More mountains will undoubtedly be discovered, but these will likely be in uncommon tumor types that have not yet been studied in depth.

The newly discovered Mut-driver genes that have been detected through genome-wide sequencing have often proved illuminating. For example, nearly half of these genes encode proteins that directly regulate chromatin through modification of histones or DNA. Examples include the histones HIST1H3B and H3F3A, as well as the proteins DNMT1 and TET1, which covalently modify DNA, EZH2, SETD2, and KDM6A, which, in turn, methylate or demethylate histones (53–57). These discoveries have profound implications for understanding the mechanistic basis of the epigenetic changes that are rampant in tumors (58). The discovery of genetic alterations in genes encoding mRNA splicing factors, such as *SF3B1* and *U2AF1* (59–61), was similarly stunning, as mutations in these genes would be expected to lead to a plethora of nonspecific cellular stresses rather than to promote specific tumor types. Another example is provided by mutations in the cooperating proteins ATRX and DAXX (62). Tumors with mutations in these genes all have a specific type of telomere elongation process termed “ALT” (for “alternative lengthening of telomeres”) (63). Though the ALT phenotype had been recognized for more than a decade, its genetic basis

was mysterious before the discovery of mutations of these genes and their perfect correlation with the ALT phenotype (64). A final example is provided by *IDH1* and *IDH2*, whose mutations have stimulated the burgeoning field of tumor metabolism (65) and have had fascinating implications for epigenetics (66, 67).

The Mut-driver genes listed in table S2A are affected by subtle mutations: base substitutions, intragenic insertions, or deletions. As noted above, Mut-driver genes can also be altered by less subtle changes, such as translocations, amplifications, and large-scale deletions. As with point mutations, it can be difficult to distinguish Mut-driver genes that are altered by these types of changes from genes that contain only passenger mutations. Genes that are not point-mutated, but are recurrently amplified (e.g., *MYC* family genes) or homozygously deleted (e.g., *MAP2K4*) and that meet other criteria (e.g., being the only gene in the amplicon or homozygously deleted region) are listed in table S2B. This adds 13 Mut-driver genes—10 oncogenes that are amplified and 3 tumor suppressor genes that are homozygously deleted—to the 125 driver genes that are affected by subtle mutations, for a total of 138 driver genes discovered to date (table S2).

Translocations provide similar challenges for driver classification. An important discovery related to this point is chromothripsis (68), a rare cataclysmic event involving one or a small number of chromosomes that results in a large number of chromosomal rearrangements. This complicates any inferences about causality, in the same way that mismatch repair deficiency compromises the interpretation of point mutations. However, for completeness, all fusion genes that have been identified in at least three independent tumors are listed in table S3. Virtually all of these genes were discovered through conventional approaches before the advent of genome-wide DNA sequencing studies, with some notable exceptions such as those described in (6) and (69). The great majority of these translocations are found in liquid tumors (leukemias and lymphomas) (table S3C) or mesenchymal tumors (table S3B) and were initially identified through karyotypic analyses. A relatively small number of recurrent fusions, the most important of which include *ERG* in prostate cancers (70) and *ALK* in lung cancers (71), have been described in more common tumors (table S3A).

Genes exist that predispose to cancer when inherited in mutant form in the germ line, but are not

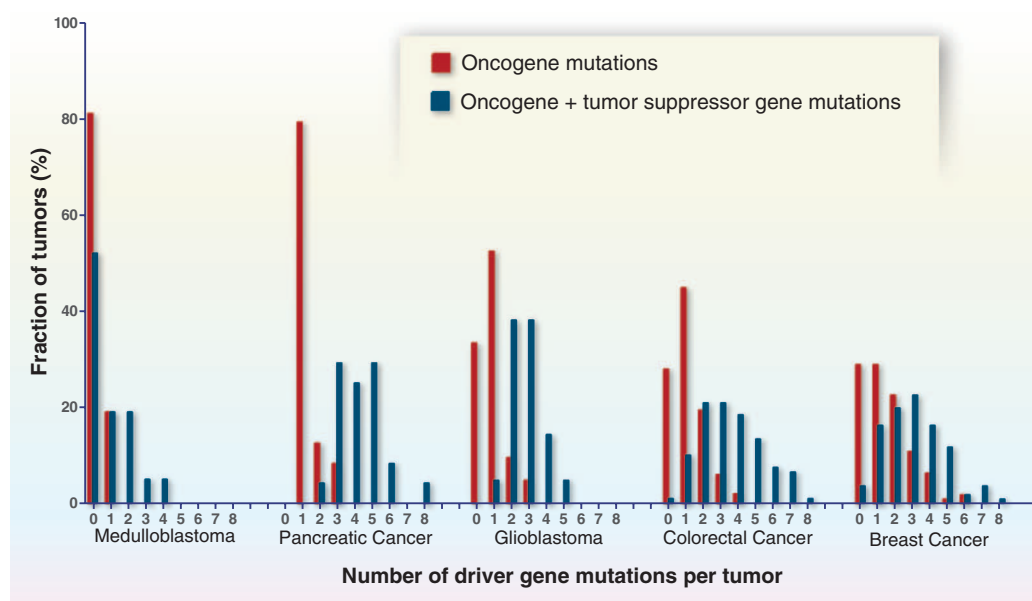


Fig. 5. Number and distribution of driver gene mutations in five tumor types. The total number of driver gene mutations [in oncogenes and tumor suppressor genes (TSGs)] is shown, as well as the number of oncogene mutations alone. The driver genes are listed in tables S2A and S2B. Translocations are not included in this figure, because few studies report translocations along with the other types of genetic alterations on a per-case basis. In the tumor types shown here, translocations affecting driver genes occur in less than 10% of samples. The published data on which this figure is based are provided in table S1E.

somatically mutated in cancer to a substantial degree. These genes generally do not confer an increase in selective growth advantage when they are abnormal, but they stimulate tumorigenesis in indirect ways (such as by increasing genetic instability, as discussed later in this Review). For completeness, these genes and the hereditary syndromes for which they are responsible are listed in table S4.

Dark Matter

Classic epidemiologic studies have suggested that solid tumors ordinarily require five to eight “hits,” now interpreted as alterations in driver genes, to develop (72). Is this number compatible with the molecular genetic data? In pediatric tumors such as medulloblastomas, the number of driver gene mutations is low (zero to two), as expected from the discussion above (Fig. 5). In common adult tumors—such as pancreatic, colorectal, breast, and brain cancers—the number of mutated driver genes is often three to six, but several tumors have only one or two driver gene mutations (Fig. 5). How can this be explained, given the widely accepted notion that tumor development and progression require multiple, sequential genetic alterations acquired over decades?

First, technical issues explain some of the “missing mutations.” Genome-wide sequencing is far from perfect, at least with the technologies available today. Some regions of the genome are not well represented because their sequences are difficult to amplify, capture, or unambiguously map to the genome (73–76). Second, there is usually a wide distribution in the number of times that a specific nucleotide in a given gene is observed in the sequence data, so some regions will not be well represented by chance factors alone (77). Finally, primary tumors contain not only neoplastic cells, but also stromal cells that dilute the signal from the mutated base, further reducing the probability of finding a mutation (78).

What fraction of mutations are missed by these three technical issues? A recent study of pancreatic cancers is informative in this regard. Biankin *et al.* used immunohistochemical and genetic analyses to select a set of primary tumor samples enriched in neoplastic cells (79). They used massively parallel sequencing to analyze the exomes of these samples, then compared their mutational data with a set of pancreatic cancer cell lines and xenografts in which mutations had previously been identified, using conventional Sanger sequencing, and confirmed to be present in the primary tumors (3, 16). Only 159 (63%) of the expected 251 driver gene mutations were identified in the primary tumors studied by next-generation sequencing alone, indicating a false-negative rate of 37%. Genome-wide studies in which the proportion of neoplastic cells within tu-

mors is not as carefully evaluated as in (79) will have higher false-negative rates. Moreover, these technical problems are exacerbated in whole-genome studies compared with exomic analyses, because the sequence coverage of the former is often lower than that of the latter (generally 30-fold in whole-genome studies versus more than 100-fold in exomic studies).

Conceptual issues also limit the number of detectable drivers. Virtually all studies, either at the whole-genome or whole-exome level, have focused on the coding regions. The reason for

interpret than the somatic mutations in cancers. The first examples of light coming to such dark matter have recently been published: Recurrent mutations in the promoter of the *TERT* gene, encoding the catalytic subunit of telomerase, have been identified and shown to activate its transcription (81, 82).

Mut-driver genes other than those listed in table S2 will undoubtedly be discovered as genome-wide sequencing continues. However, based on the trends noted above, most of the Mut-driver genes will likely be mountains in

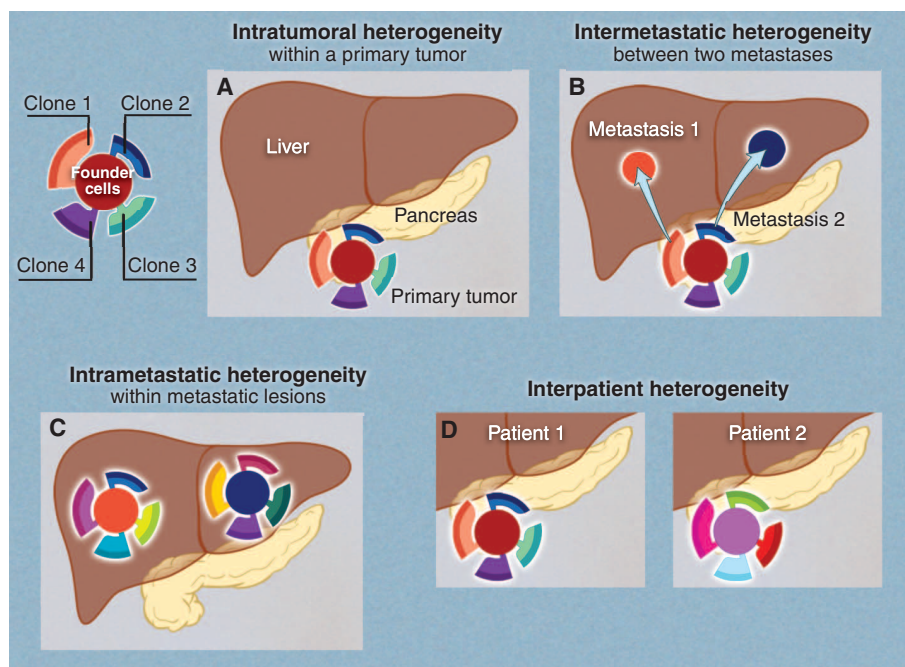


Fig. 6. Four types of genetic heterogeneity in tumors, illustrated by a primary tumor in the pancreas and its metastatic lesions in the liver. Mutations introduced during primary tumor cell growth result in clonal heterogeneity. At the top left, a typical tumor is represented by cells with a large fraction of the total mutations (founder cells) from which subclones are derived. The differently colored regions in the subclones represent stages of evolution within a subclone. (A) Intratumoral: heterogeneity among the cells of the primary tumor. (B) Intermetastatic: heterogeneity among different metastatic lesions in the same patient. In the case illustrated here, each metastasis was derived from a different subclone. (C) Intrametastatic: heterogeneity among the cells of each metastasis develops as the metastases grow. (D) Interpatient: heterogeneity among the tumors of different patients. The mutations in the founder cells of the tumors of these two patients are almost completely distinct (see text).

this is practical; it is difficult enough to identify driver gene mutations when they qualitatively alter the sequence of the encoded protein. Trying to make sense of intergenic or intronic mutations is much more difficult. Based on analogous studies of the identifiable mutations in patients with monogenic diseases, more than 80% of mutations should be detectable through analysis of the coding regions (80). However, this still leaves some mutations as unidentifiable “dark matter,” even in the germline genomes of heritable cases, which are usually easier to in-

terpret than the somatic mutations in cancers. rare tumor types or small hills in common tumor types; thus, these genes are unlikely to account for the bulk of the presumptive dark matter. Other types of dark matter can be envisioned, however. Copy-number alterations are ubiquitous in cancers, at either the whole-chromosome or subchromosomal levels. These alterations could subtly change the expression of their driver genes. Recent studies have suggested that the loss of one copy of chromosomes containing several tumor suppressor genes, each plausibly connected to neoplasia but not altered by

mutation, may confer a selective growth advantage (83, 84).

The most obvious source of dark matter is in Epi-driver genes. Human tumors contain large numbers of epigenetic changes affecting DNA or chromatin proteins. For example, a recent study of colorectal cancers showed that more than 10% of the protein-coding genes were differentially methylated when compared with normal colorectal epithelial cells (85). Some of these changes (i.e., those in Epi-driver genes) are likely to provide a selective growth advantage (86, 87). For example, epigenetic silencing of *CDK2NA* and *MLH1* is much more common than mutational inactivation of either of these two well-recognized driver genes (85). However, there is a critical difference between a genetic and an epigenetic change in a gene. Unlike the sequence of a gene in a given individual, methylation is plastic, varying with cell type, developmental stage, and patient age (21). The methylation state of the normal precursor cells that initiate tumorigenesis is unknown; these cells, such as normal stem cells, may represent only a tiny fraction of the cells in a normal organ. This plasticity also means that methylation can change under microenvironmental cues, such as those associated with low nutrient concentrations or abnormal cell contacts. It is therefore difficult to know whether specific epigenetic changes observed in cancer cells reflect, rather than contribute to, the neoplastic state. Criteria for distinguishing epigenetic changes that exert a selective growth advantage from those that do not (passenger epigenetic changes) have not yet been formulated. Given that Epi-driver genes are likely to compose a major component of the dark matter, further research on this topic is essential (58).

Genetic Heterogeneity

The mutations depicted in Fig. 1 are clonal; that is, they are present in the majority of the neoplastic cells in the tumors. But additional, subclonal (i.e., heterogeneous within the tumor) mutations are important for understanding tumor evolution. Four types of genetic heterogeneity are relevant to tumorigenesis (Fig. 6):

1) Intratumoral: heterogeneity among the cells of one tumor. This type of heterogeneity has been recognized for decades. For example, it is rare to see a cytogenetic study of a solid tumor in which all of the tumor cells display the same karyotype (88). The same phenomenon has been noted for individual genes [e.g., (89)] and more recently has been observed throughout the genome (16, 90–96). This kind of heterogeneity must exist: Every time a normal (or tumor) cell divides, it acquires a few mutations, and the number of mutations that distinguish any two cells simply marks the time from their last common ancestor (their founder cell). Cells at the opposite ends of large tumors will be spa-

tially distinct and, in general, will display more differences than neighboring cells (16). This phenomenon is analogous to speciation, wherein organisms on different islands are more likely to diverge from one another than are organisms on the same island.

In studies that have evaluated intratumoral heterogeneity by genome-wide sequencing, the majority of somatic mutations are present in all tumor cells. These mutations form the trunk of the somatic evolutionary tree. What is the importance of the mutations in the branches (i.e., those that are not shared by all tumor cells)? From a medical perspective, these mutations are often meaningless because the primary tumors are surgically removed. How much heterogeneity existed in the various branches before surgery is not important. However, this heterogeneity provides the seeds for intermetastatic heterogeneity, which is of great clinical importance.

2) Intermetastatic: heterogeneity among different metastatic lesions of the same patient. The vast majority of cancer patients die because their tumors were not removed before metastasis to surgically inaccessible sites, such as the liver, brain, lung, or bone. Patients who relapse with a single metastatic lesion can often still be cured by surgery or radiotherapy, but single metastases are the exception rather than the rule. A typical patient on a clinical trial has a dozen or more metastatic lesions large enough to be visualized by imaging, and many more that are smaller. If each of the metastatic lesions in a single patient was founded by a cell with a very different genetic constitution, then chemotherapeutic cures would be nearly impossible to achieve: Eradicating a subset of the metastatic lesions in a patient will not be adequate for long-term survival.

How much heterogeneity is there among different metastatic lesions? In short, a lot. It is not uncommon for one metastatic lesion to have 20 clonal genetic alterations not shared by other metastases in the same patient (16, 97). Because they are clonal, these mutations occurred in the founder cell of the metastasis; that is, the cell that escaped from the primary tumor and multiplied to form the metastasis. The founder cell for each metastasis is present in different, geographically distinct areas of the primary tumors, as expected (16).

This potentially disastrous situation is tempered by the fact that the heterogeneity appears largely confined to passenger gene mutations. In most of the studies documenting heterogeneity in malignancies, the Mut-driver genes are present in the trunks of the trees, though exceptions have been noted (95). These findings are consistent with the idea, discussed above, that the genetic alterations required for metastasis were present (i.e., selected for) before metastasis actually occurred. The data are also

consistent with the observation that in patients responsive to targeted agents, the response is often seen in all metastatic lesions rather than just a small subset (98).

3) Intrametastatic: heterogeneity among the cells of an individual metastasis. Each metastasis is established by a single cell (or small group of cells) with a set of founder mutations. As it grows, the metastasis acquires new mutations with each cell division. Though the founder mutations may make the lesion susceptible to antitumor agents, the new mutations provide the seeds for drug resistance. Unlike primary tumors, the metastatic lesions generally cannot be removed by surgery and must be treated with systemic therapies. Patients with complete responses to targeted therapies invariably relapse. Most of the initial lesions generally recur, and the time frame at which they recur is notably similar. This time course can be explained by the presence of resistance mutations that existed within each metastasis before the onset of the targeted therapy (99–102). Calculations show that any metastatic lesion of a size visible on medical imaging has thousands of cells (among the billions present) that are already resistant to virtually any drug that can be imagined (99, 101, 102). Thus, recurrence is simply a matter of time, entirely predictable on the basis of known mutation frequencies and tumor cell growth rates. This “fait accompli” can be circumvented, in principle, by treatment with multiple agents, as it is unlikely that a single tumor cell will be resistant to multiple drugs that act on different targets.

4) Interpatient: heterogeneity among the tumors of different patients. This type of heterogeneity has been observed by every oncologist; no two cancer patients have identical clinical courses, with or without therapy. Some of these differences could be related to host factors, such as germline variants that determine drug half-life or vascular permeability to drugs or cells, and some could be related to nongenetic factors (103). However, much of this interpatient heterogeneity is probably related to somatic mutations within tumors. Though several dozen somatic mutations may be present in the breast cancers from two patients, only a small number are in the same genes, and in the vast majority of cases, these are the Mut-driver genes (1, 104, 105). Even in these driver genes, the actual mutations are often different. Mutations altering different domains of a protein would certainly not be expected to have identical effects on cellular properties, as experimentally confirmed (106). Though it may seem that different mutations in adjacent codons would have identical effects, detailed studies of large numbers of patients have shown that this need not be the case. For example, a Gly¹²→Asp¹² (G12D) mutation of *KRAS* does not have the same clinical implications as a G13D mutation of the same gene (107). Interpatient heterogeneity has always been one of the major obstacles

to designing uniformly effective treatments for cancer. Efforts to individualize treatments based on knowledge of the genomes of cancer patients are largely based on an appreciation of this heterogeneity.

Signaling Pathways in Tumors

The immense complexity of cancer genomes that could be inferred from the data described above is somewhat misleading. After all, even advanced tumors are not completely out of control, as evidenced by the dramatic responses to agents that target mutant *BRAF* in melanomas (108) or mutant *ALK* in lung cancers (109). Albeit transient, these responses mean that interference with even a single mutant gene product is sufficient to stop cancer in its tracks, at least transiently. How can the genomic complexity of cancer be reconciled with these clinical observations?

Two concepts bear on this point. The first, mentioned above, is that >99.9% of the alterations in tumors (including point mutations, copy-number alterations, translocations, and epigenetic changes distributed throughout the genome, not just in the coding regions) are immaterial to neoplasia. They are simply passenger changes that mark the time that has elapsed between successive clonal expansions. Normal cells also undergo genetic alterations as they divide, both at the nucleotide and chromosomal levels. However, normal cells are programmed to undergo

cell death in response to such alterations, perhaps as a protective mechanism against cancer. In contrast, cancer cells have evolved to tolerate genome complexity by acquiring mutations in genes such as *TP53* (110). Thus, genomic complexity is, in part, the result of cancer, rather than the cause.

To appreciate the second concept, one must take the 30,000-foot view. A jungle might look chaotic at ground level, but the aerial view shows a clear order, with all the animals gathering at the streams at certain points in the day, and all the streams converging at a river. There is order in cancer, too. Mutations in all of the 138 driver genes listed in table S2 do one thing: cause a selective growth advantage, either directly or indirectly. Moreover, there appears to be only a limited number of cellular signaling pathways through which a growth advantage can be incurred (Fig. 7 and table S5).

All of the known driver genes can be classified into one or more of 12 pathways (Fig. 7). The discovery of the molecular components of these pathways is one of the greatest achievements of biomedical research, a tribute to investigators working in fields that encompass biochemistry, cell biology, and development, as well as cancer. These pathways can themselves be further organized into three core cellular processes:

1) Cell fate: Numerous studies have demonstrated the opposing relationship between cell division and differentiation, the arbiters of cell fate. Dividing cells that are responsible for populating normal tissues (stem cells) do not differentiate, and vice versa. Regenerative medicine is based on this distinction, predicated on ways to get differentiated cells to de-differentiate into stem cells, then forcing the stem cells to differentiate into useful cell types for transplantation back into the patient. Many of the genetic alterations in cancer abrogate the precise balance between differentiation and division, favoring the latter. This causes a selective growth advantage, because differentiating cells eventually die or become quiescent. Pathways that function through this process include APC, HH, and NOTCH, all of which are well known to control cell fate in organisms ranging from worms to mammals (111). Genes encoding chromatin-modifying enzymes can also be included in this category. In normal development, the heritable switch from division to differentiation is not determined by mutation, as it is in cancer, but rather

by epigenetic alterations affecting DNA and chromatin proteins. What better way to subvert this normal mechanism for controlling tissue architecture than to debilitate the epigenetic modifying apparatus itself?

2) Cell survival: Though cancer cells divide abnormally because of cell-autonomous alterations, such as those controlling cell fate, their surrounding stromal cells are perfectly normal and do not keep pace. The most obvious ramification of this asymmetry is the abnormal vasculature of tumors. As opposed to the well-ordered network of arteries, veins, and lymphatics that control nutrient concentrations in normal tissues, the vascular system in cancers is tortuous and lacks uniformity of structure (112, 113). Normal cells are always within 100 μm of a capillary, but this is not true for cancer cells (114). As a result, a cancer cell acquiring a mutation that allows it to proliferate under limiting nutrient concentrations will have a selective growth advantage, thriving in environments in which its sister cells cannot. Mutations of this sort occur, for example, in the *EGFR*, *HER2*, *FGFR2*, *PDGFR*, *TGFBR2*, *MET*, *KIT*, *RAS*, *RAF*, *PIK3CA*, and *PTEN* genes (table S2A). Some of these genes encode receptors for the growth factors themselves, whereas others relay the signal from the growth factor to the interior of the cell, stimulating growth when activated (115, 116). For instance, mutations in *KRAS* or *BRAF* genes confer on cancer cells the ability to grow in glucose concentrations that are lower than those required for the growth of normal cells or of cancer cells that do not have mutations in these genes (117, 118). Progression through the cell cycle (and its antithesis, apoptosis) can be directly controlled by intracellular metabolites, and driver genes that directly regulate the cell cycle or apoptosis, such as *CDKN2A*, *MYC*, and *BCL2*, are often mutated in cancers. Another gene whose mutations enhance cell survival is *VHL*, the product of which stimulates angiogenesis through the secretion of vascular endothelial growth factor. What better way to provision growth factors to a rogue tumor than to lure the unsuspecting vasculature to its hideout?

3) Genome maintenance: As a result of the exotic microenvironments in which they reside, cancer cells are exposed to a variety of toxic substances, such as reactive oxygen species. Even without microenvironmental poisons, cells make mistakes while replicating their DNA or during division (119, 120), and checkpoints exist to either slow down such cells or make them commit suicide (apoptosis) under such circumstances (110, 121, 122). Although it is good for the organism to remove these damaged cells, tumor cells that can survive the damage will, by definition, have a selective growth advantage. Therefore, it is not surprising that genes whose mutations abrogate these checkpoints, such as *TP53* and *ATM*, are mutated in cancers



Fig. 7. Cancer cell signaling pathways and the cellular processes they regulate. All of the driver genes listed in table S2 can be classified into one or more of 12 pathways (middle ring) that confer a selective growth advantage (inner circle; see main text). These pathways can themselves be further organized into three core cellular processes (outer ring). The publications on which this figure is based are provided in table S5.

(123). Defects in these genes can also indirectly confer a selective growth advantage by allowing cells that have a gross chromosomal change favoring growth, such as a translocation or an extra chromosome, to survive and divide. Analogously, genes that control point mutation rates, such as *MLH1* or *MSH2*, are mutated in cancers (table S2A) or in the germ line of patients predisposed to cancers (table S4) because they accelerate the acquisition of mutations that function through processes that regulate cell fate or survival. What better way to promote cancer than by increasing the rate of occurrence of the mutations that drive the process?

Because the protein products of genes regulating cell fate, cell survival, and genome maintenance often interact with one another, the pathways within them overlap; they are not as discrete as might be inferred from the description above. However, grouping genes into pathways makes perfect sense from a genetics standpoint. Given that cancer is a genetic disease, the principles of genetics should apply to its pathogenesis. When performing a conventional mutagenesis screen in bacteria, yeast, fruit flies, or worms, one expects to discover mutations in several different genes that confer similar phenotypes. The products of these genes often interact with one another and define a biochemical or developmental pathway. Therefore, it should not be surprising that several different genes can result in the same selective growth advantage for cancer cells and that the products of these genes interact. The analogy between cancer pathways and biochemical or developmental pathways in other organisms goes even deeper: The vast majority of our knowledge of the function of driver genes has been derived from the study of the pathways through which their homologs work in nonhuman organisms. Though the functions are not identical to those in human cells, they are highly related and have provided the starting point for analogous studies in human cells.

Recognition of these pathways also has important ramifications for our ability to understand interpatient heterogeneity. One lung cancer might have an activating mutation in a receptor for a stimulatory growth factor, making it able to grow in low concentrations of epidermal growth factor (EGF). A second lung cancer might have an activating mutation in *KRAS*, whose protein product normally transmits the signal from the epidermal growth factor receptor (EGFR) to other cell signaling molecules. A third lung cancer might have an inactivating mutation in *NF1*, a regulatory protein that normally inactivates the *KRAS* protein. Finally, a fourth lung cancer might have a mutation in *BRAF*, which transmits the signal from *KRAS* to downstream kinases (Fig. 8). One would predict that mutations in the various components of a single pathway would be mutually exclusive—that is, not occurring in the

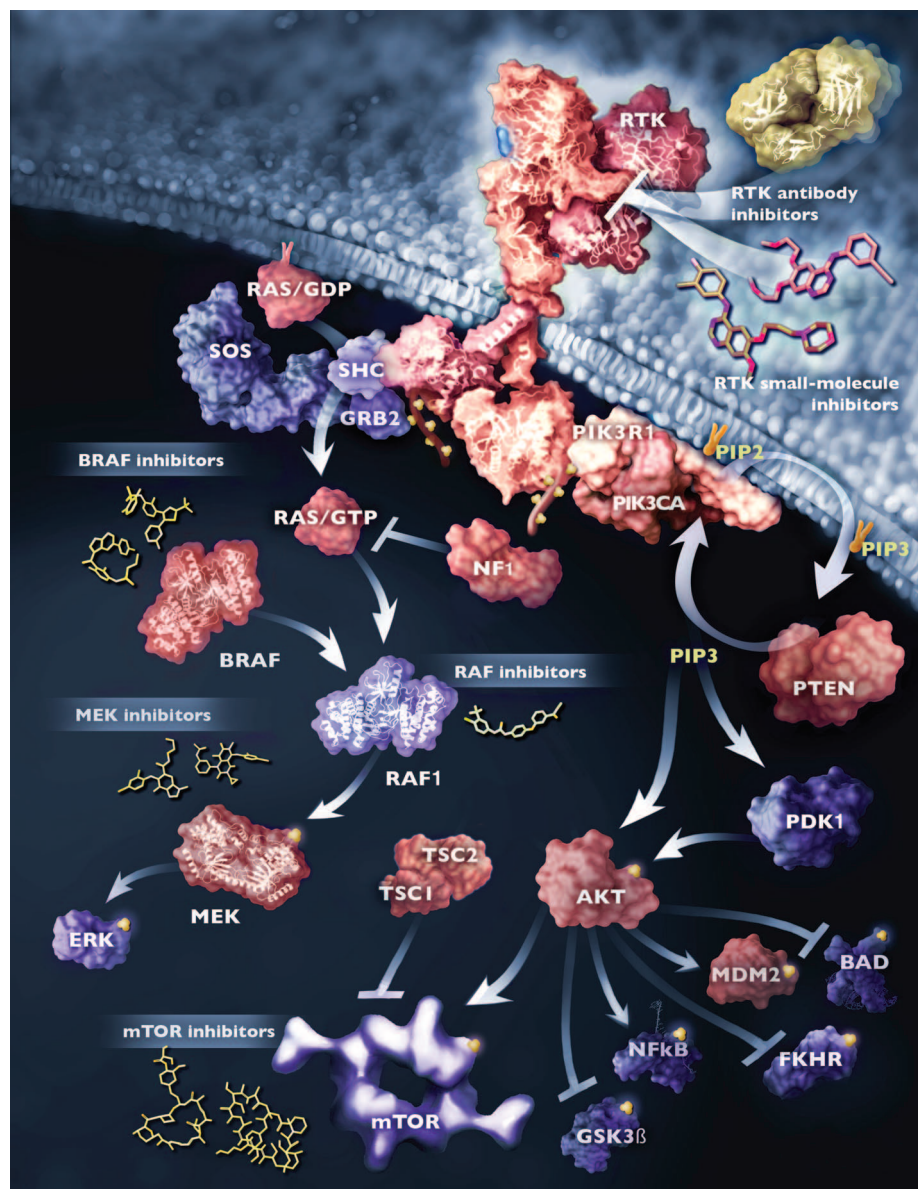


Fig. 8. Signal transduction pathways affected by mutations in human cancer. Two representative pathways from Fig. 7 (RAS and PI3K) are illustrated. The signal transducers are color coded: red indicates protein components encoded by the driver genes listed in table S2; yellow balls denote sites of phosphorylation. Examples of therapeutic agents that target some of the signal transducers are shown. RTK, receptor tyrosine kinase; GDP, guanosine diphosphate; MEK, MAPK kinase; ERK, extracellular signal-regulated kinase; NFkB, nuclear factor κ B; mTOR, mammalian target of rapamycin.

same tumor—and this has been experimentally confirmed (124, 125). Apart from being intellectually satisfying, knowledge of these pathways has implications for cancer therapy, as discussed in the next section.

A Perspective on Genome-Based Medicine in Oncology

Opportunities

Though cancer genome sequencing is a relatively new endeavor, it has already had an impact on the

clinical care of cancer patients. The recognition that certain tumors contain activating mutations in driver genes encoding protein kinases has led to the development of small-molecule inhibitor drugs targeting those kinases.

Representative examples of this type of genome-based medicine include the use of EGFR kinase inhibitors to treat cancers with *EGFR* gene mutations (126), the aforementioned anaplastic lymphoma kinase (ALK) inhibitors to treat cancers with *ALK* gene translocations (109), and specific inhibitors of mutant BRAF

to treat cancers with *BRAF* mutations (108). Before instituting treatment with such agents, it is imperative to determine whether the cancer harbors the mutations that the drug targets. Only a small fraction of lung cancer patients have *EGFR* gene mutations or *ALK* gene translocations, and only these patients will respond to the drugs. Treating lung cancer patients without these particular genetic alterations would be detrimental, as such patients would develop the toxic side effects of the drugs while their tumors progressed.

A second type of genome-based medicine focuses on the side effects and metabolism of the therapeutic agents, rather than the genetic alterations they target. At present, the dose of cancer drugs given to patients is based on the patients' size (body weight or surface area). But the therapeutic ratio of cancer drugs (ratio of the concentration that causes side effects to the concentration required to kill tumor cells) is generally low, particularly for conventional (nontargeted) therapeutic agents. Small changes in circulating concentrations of these drugs can make the difference between substantial tumor regression and intolerable side effects. Interrogation of the germline status of the genes encoding drug-metabolizing enzymes could substantially improve the outcomes of treatment by informing drug dosing (127). Optimally, this genome interrogation would be accompanied by pharmacokinetic measurements of drug concentrations in each patient. The additional cost of such analyses would be small compared with the exorbitant costs of new cancer therapies—for recently approved drugs, the cost is estimated to be \$200,000 to \$300,000 per quality life year produced (128).

Challenges

One challenge of genome-based medicine in oncology is already apparent from the opportunities described above: All of the clinically approved drugs that target the products of genetically altered genes are directed against kinases. One reason for this is that kinases are relatively easy to target with small molecules and have been extensively studied at the biochemical, structural, and physiologic levels (129). But another reason has far deeper ramifications. The vast majority of drugs on the market today, for cancer or other diseases, inhibit the actions of their protein targets. This inhibition occurs because the drugs interfere with the protein's enzymatic activity (such as the phosphorylation catalyzed by kinases) or with the binding of the protein to a small ligand (such as with G protein-coupled receptors). Only 31 of the oncogenes listed in tables S2 and S3 have enzymatic activities that are targetable in this manner. Many others participate in protein complexes, involving large interfaces and numerous weak interactions. Inhibiting the function of such proteins

with small drugs is notoriously difficult because small compounds can only inhibit one of these interactions (130, 131).

Though one can at least imagine the development of drugs that inhibit nonenzymatic protein functions, the second challenge evident from table S2 poses even greater difficulties: A large fraction of the Mut-driver genes encode tumor suppressors. Drugs generally interfere with protein function; they cannot, in general, replace the function of defective genes such as those resulting from mutations in tumor suppressor genes. Unfortunately, tumor suppressor gene-inactivating mutations predominate over oncogene-activating mutations in the most common solid tumors: Few individual tumors contain more than one oncogene mutation (Fig. 5).

The relatively small number of oncogene mutations in tumors is important in light of the intrametastatic heterogeneity described earlier. To circumvent the inevitable development of resistance to targeted therapies, it will likely be necessary to treat patients with two or more drugs. The probability that a single cancer cell within a large metastatic lesion will be resistant to two agents that target two independent pathways is exponentially less than the probability that the cell will be resistant to a single agent. However, if the cancer cell does not contain more than one targetable genetic alteration (i.e., an oncogene mutation), then this combination strategy is not feasible.

Given the paucity of oncogene alterations in common solid tumors and these principles, can

targeted therapeutic approaches ever be expected to induce long-term remissions, even cures, rather than the short-term remissions now being achieved? The saviors are pathways; every tumor suppressor gene inactivation is expected to result in the activation of some growth-promoting signal downstream of the pathway. An example is provided by *PTEN* mutations: Inactivation of the tumor suppressor gene *PTEN* results in activation of the AKT kinase (Fig. 8). Similarly, inactivation of the tumor suppressor gene *CDKN2A* results in activation of kinases, such as cyclin-dependent kinase 4, that promote cell cycle traverse (132). Furthermore, inactivation of tumor suppressor gene *APC* results in constitutive activity of oncogenes such as *CTNNB1* and *CMYC* (133–135).

We believe that greater knowledge of these pathways and the ways in which they function is the most pressing need in basic cancer research. Successful research on this topic should allow the development of agents that target, albeit indirectly, defective tumor suppressor genes. Indeed, there are already examples of such indirect targeting. Inactivating mutations of the tumor suppressor genes *BRCA1* or *BRCA2* lead to activation of downstream pathways required to repair DNA damage in the absence of BRCA function. Thus, cancer cells with defects in *BRCA1* or *BRCA2* are more susceptible to DNA damaging agents or to drugs that inhibit enzymes that facilitate the repair of DNA damage such as PARP [poly(adenosine diphosphate–ribose) polymerase] (136). PARP inhibitors have shown

Box 2. Highlights

1. Most human cancers are caused by two to eight sequential alterations that develop over the course of 20 to 30 years.
2. Each of these alterations directly or indirectly increases the ratio of cell birth to cell death; that is, each alteration causes a selective growth advantage to the cell in which it resides.
3. The evidence to date suggests that there are ~140 genes whose intragenic mutations contribute to cancer (so-called Mut-driver genes). There are probably other genes (Epi-driver genes) that are altered by epigenetic mechanisms and cause a selective growth advantage, but the definitive identification of these genes has been challenging.
4. The known driver genes function through a dozen signaling pathways that regulate three core cellular processes: cell fate determination, cell survival, and genome maintenance.
5. Every individual tumor, even of the same histopathologic subtype as another tumor, is distinct with respect to its genetic alterations, but the pathways affected in different tumors are similar.
6. Genetic heterogeneity among the cells of an individual tumor always exists and can impact the response to therapeutics.
7. In the future, the most appropriate management plan for a patient with cancer will be informed by an assessment of the components of the patient's germline genome and the genome of his or her tumor.
8. The information from cancer genome studies can also be exploited to improve methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortality.

encouraging results in clinical trials when used in patients whose tumors have inactivating mutations of BRCA genes (137).

Further progress in this area will require more detailed information about the signaling pathways through which cancer genes function in human cancer cells, as well as in model organisms. One of the lessons of molecular biology over the past two decades is that pathway functions are different, depending on the organism, cell type, and precise genetic alterations in that cell (138). A pertinent example of this principle is provided by results of treatment with drugs inhibiting mutant BRAF kinase activity. In the majority of patients with melanomas harboring (V600E; V, Val; E, Glu) mutations in the *BRAF* gene, these drugs induce dramatic (though transient) remissions (108). But the same drugs have no therapeutic effect in colorectal cancer patients harboring the identical *BRAF* mutations (139). This observation has been attributed to the expression of EGFR, which occurs in some colorectal cancers but not in melanoma and is thought to circumvent the growth-inhibitory effects of the BRAF inhibitors. With this example in mind, no one should be surprised that a new drug that works well in an engineered tumor in mice fails in human trials; the organism is different, the cell type is usually different, and the precise genetic constitutions are always different. The converse of this statement—that a drug that fails in animal trials will not necessarily fail in human trials—has important practical consequences. In our view, if the biochemical and conceptual bases for a drug's actions are solid and the drug is shown to be safe in animals, then a human trial may be warranted, even if it does not shrink tumors in mice.

Genome-Based Medicines of the Future

Cancer genomes can also be exploited for the development of more effective immunotherapies. As noted above, typical solid tumors contain 30 to 70 mutations that alter the amino acid sequences of the proteins encoded by the affected genes. Each of these alterations is foreign to the immune system, as none have been encountered during embryonic or postnatal life. Therefore, these alterations, in principle, provide a “holy grail” for tumor immunology: truly tumor-specific antigens. These antigens could be incorporated into any of the numerous platforms that already exist for the immunotherapy of cancer. These include administration of vaccines containing the mutant peptide, viruses encoding the mutant peptides on their surfaces, dendritic cells presenting the mutated peptide, and antibodies or T cells with reactivity directed against the mutant peptides (140).

To realize these sorts of therapeutics, several conditions must be met. First, the mutant protein must be expressed. As cancer cells generally express about half of the proteins that are encoded

by the human genome (141), this condition is not limiting. Second, as most proteins affected by mutations are intracellular, these mutations will not be visible to the immune system unless the mutant residue is presented in the context of a human leukocyte antigen (HLA) protein. Based on *in silico* analyses of binding affinities, it has been estimated that a typical breast or colorectal cancer contains 7 to 10 mutant proteins that can bind to an individual patient's HLA type (142). These theoretical predictions have recently gained experimental support. Studies of mouse tumors have identified mutant genes and shown that the corresponding peptides can induce antitumor immunity when administered as vaccines (143). Moreover, clinical trials of brain cancer patients immunized against a mutant peptide have yielded encouraging results (144).

As with all cancer therapies that are attractive in concept, obstacles abound in practice. If a tumor expresses a mutant protein that is recognizable as foreign, why has the host immune system not eradicated that tumor already? Indeed, immunoediting in cancers has been shown to exist, resulting in the down-regulation or absence of mutant epitopes that should have, and perhaps did, elicit an immune response during tumor development (145, 146). Additionally, tumors can lose immunogenicity through a variety of genetic alterations, thereby precluding the presentation of epitopes that would otherwise be recognized as foreign (147). Though these theoretical limitations are disheartening, recent studies on immune regulation in humans portend cautious optimism (148, 149).

Other Ways to Reduce Morbidity and Mortality Through Knowledge of Cancer Genomics

When we think about eradicating cancer, we generally think about curing advanced cases—those that cannot be cured by surgery alone because they have already metastasized. This is a curious way of thinking about this disease. When we think of cardiovascular or infectious diseases, we first consider ways to prevent them rather than drugs to cure their most advanced forms. Today, we are in no better position to cure polio or massive myocardial infarctions than we were a thousand years ago. But we can prevent these diseases entirely (vaccines), reduce incidence (dietary changes, statins), or mitigate severity (stents, thrombolytic agents) and thereby make a major impact on morbidity and mortality.

This focus on curing advanced cancers might have been reasonable 50 years ago, when the molecular pathogenesis of cancers was mysterious and when chemotherapeutic agents against advanced cancers were showing promise. But this mindset is no longer acceptable. We now know precisely what causes cancer: a sequential series of alterations in well-defined genes that

alter the function of a limited number of pathways. Moreover, we know that this process takes decades to develop and that the incurable stage, metastasis, occurs only a few years before death. In other words, of the one million people that will die from cancer this year, the vast majority will die only because their cancers were not detected in the first 90% of the cancers' lifetimes, when they were amenable to the surgeons' scalpel.

This new knowledge of cancer (Box 2) has reinvigorated the search for cures for advanced cancers, but has not yet permeated other fields of applied cancer research. A common and limited set of driver genes and pathways is responsible for most common forms of cancer (table S2); these genes and pathways offer distinct potential for early diagnosis. The genes themselves, the proteins encoded by these genes, and the end products of their pathways are, in principle, detectable in many ways, including analyses of relevant body fluids, such as urine for genitourinary cancers, sputum for lung cancers, and stool for gastrointestinal cancers (150). Equally exciting are the possibilities afforded by molecular imaging, which not only indicate the presence of a cancer but also reveal its precise location and extent. Additionally, research into the relationship between particular environmental influences (diet and lifestyle) and the genetic alterations in cancer is sparse, despite its potential for preventative measures.

The reasons that society invests so much more in research on cures for advanced cancers than on prevention or early detection are complex. Economic issues play a part: New drugs are far more lucrative for industry than new tests, and large individual costs for treating patients with advanced disease have become acceptable, even in developing countries (151). From a technical standpoint, the development of new and improved methods for early detection and prevention will not be easy, but there is no reason to assume that it will be more difficult than the development of new therapies aimed at treating widely metastatic disease.

Our point is not that strenuous efforts to develop new therapies for advanced cancer patients should be abandoned. These will always be required, no matter our arsenal of early detection or preventative measures. Instead, we are suggesting that “plan A” should be prevention and early detection, and “plan B” (therapy for advanced cancers) should be necessary only when plan A fails. To make plan A viable, government and philanthropic organizations must dedicate a much greater fraction of their resources to this cause, with long-term considerations in mind. We believe that cancer deaths can be reduced by more than 75% in the coming decades (152), but that this reduction will only come about if greater efforts are made toward early detection and prevention.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6127/1546/DC1
Tables S1 to S5

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REVIEW

Diagnostic Cancer Genome Sequencing and the Contribution of Germline Variants

O. Kilpivaara and L. A. Aaltonen*

Whole-genome sequencing (WGS) is revolutionizing medical research and has the potential to serve as a powerful and cost-effective diagnostic tool in the management of cancer. We review the progress to date in the use of WGS to reveal how germline variants and mutations may be associated with cancer. We use colorectal cancer as an example of how the current level of knowledge can be translated into predictions of predisposition. We also address challenges in the clinical implementation of the variants in germline DNA identified through cancer genome sequencing. We call for the international development of standards to facilitate the clinical use of germline information arising from diagnostic cancer genome sequencing.

Technological advances have enabled turnaround times of less than a week between sample collection from a given individual and the availability of the whole-genome sequence (WGS) of that individual in research settings (1). With these data in hand, the variation within an individual—ranging from large structural variants (such as copy number changes) to small-scale variations [such as insertions, deletions, and single-nucleotide variants (SNVs)]—can be analyzed comprehensively. Increasingly cost-effective WGS approaches have been developed, and commercial WGS is available for less than \$10,000 (www.genome.gov/sequencing-costs, accessed 11 February 2013).

DNA-based testing has become routine in modern health care. For example, in Finland—in a population of 5.4 million—approximately 100,000 such tests were performed in 2012 (2). WGS will soon facilitate most medical genetic tests because of its ability to analyze multiple genetic markers—in principle, all bases in the human genome; in practice, about 90% of them (3)—at one time. However, a transition from targeted DNA analysis to WGS requires more than the simple technical ability to do so. Housing the data is a challenge, not merely because of storage space but for reasons of data security and confidentiality (4). After robust variant calling to exclude technical artifacts, which itself is a far from trivial task (5–7), the interpretation of a given individual's results requires sophisticated skills and knowledge of well-documented variants. It will take time before a robust database is available in the public domain, although early

efforts are under way (8). Furthermore, individuals with a genetic predisposition to cancer exhibit variation in their probability of developing tumors because of genotypic differences in the degree of penetrance (the level in which a specific genotype affects the phenotype of an indi-

vidual) and heritability (the extent to which a phenotype is due to genetic factors). Clients also need to be appropriately counseled and educated prior to WGS in the event that unexpected genetic information is identified that is unrelated to the condition that triggered the testing. However, health care professionals capable of performing these tasks are few, and their education is a long-term process. Because of the novelty of the technology behind WGS, governmental guidelines are not well developed; most medical facilities have adopted a “wait-and-see” tactic that has delayed the implementation of WGS as a clinical tool.

Associating WGS-Identified Germline Mutations with Cancer

In cancer patients, the object of WGS is to obtain and compare information about cancer and germline DNA (9–11) (Fig. 1). Analyses of these data provide a catalog of somatic variants, present in the tumor genome but not in the normal tissue DNA, and a catalog of germline variants. The goal of these analyses is to reveal a drug target in the examined cancer, facilitating selection of therapy (12, 13). However, little attention has been paid to the germline variants despite their potential to provide health-related information, for example, on cancer susceptibility and drug metabolism (14).

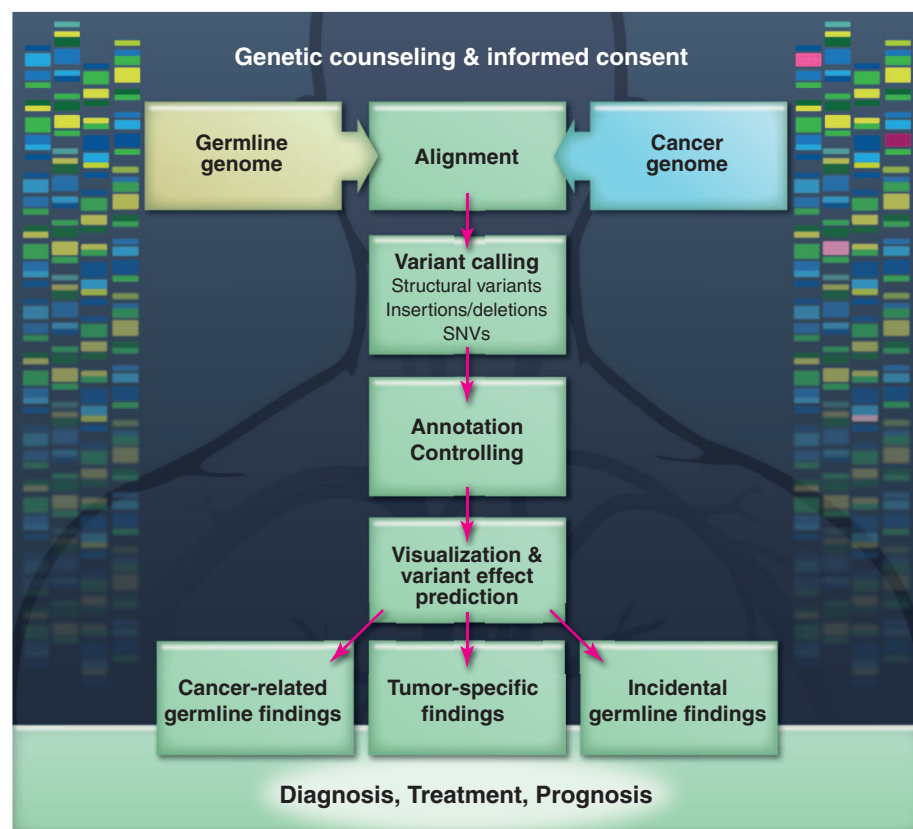


Fig. 1. Flow chart of the genome analysis for a cancer patient. The process needs to be based on appropriate genetic counseling. The results comprise three parts: somatic findings in the tumor, cancer-related findings in the germline, and incidental findings in the germline.

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Cost-effective approaches to sequencing of the whole exome (i.e., the coding regions of the genome) became available in 2009 (15), and the method has been applied to predisposition studies in several cancer types (16–18). The first study in which the whole genome of tumor and normal cells was sequenced examined a patient with acute myeloid leukemia (AML) (19). This study identified eight nonsynonymous somatic SNVs in the malignant cells in addition to two previously described AML-associated variations [somatic insertions in *fms-related tyrosine kinase 3* (*FLT3*) and *nucleophosmin* (*NPM1*)]. The patient succumbed to the disease 24 months after the initial diagnosis. However, the study established WGS as a way to discover mutations that may suggest

targeted therapies. Although much of the subsequent work on cancer using WGS has focused on somatic changes in the tumor, progress in identifying germline variants associated with susceptibility to cancer has been made, as demonstrated by the following examples presented chronologically.

TP53 (tumor protein p53)

Carriers of *TP53* mutations have an approximately 90% lifetime risk of developing cancer—specifically Li-Fraumeni syndrome (20)—often at an early age (21). WGS can identify novel variants in known cancer susceptibility genes, such as in the case of a 37-year-old patient with a history of breast and ovarian cancer followed by therapy-

related AML (22). In this case, WGS revealed a 3-kb deletion in the *TP53* gene in her germline.

ATM (ataxia telangiectasia mutated)

Deleterious mutations in *ATM* were identified via whole-genome and whole-exome sequencing in two families with hereditary pancreatic cancer (23). In this study, *ATM* variants were identified as the most promising candidates among 156 inactivating variants that previously were not present in single-nucleotide polymorphism (SNP) databases, and that were heterozygous and segregating in all affected family members. The study identified *ATM* as a pancreatic cancer predisposition gene, thereby demonstrating the power of WGS to identify the basis of familial cancer syndromes.

Table 1. Disease-specific predisposition genes to be evaluated in WGS data of a colorectal cancer patient. Interpretation and counseling (both pre- and posttest) for this narrow set of data are not trivial and highlight the challenges in the vastly more extensive open-ended WGS testing.

High-penetrance colon cancer syndrome	Predisposition gene(s)	Gene function
Lynch syndrome	<i>mutL homolog 1 (MLH1)</i> <i>mutS homolog 2 (MSH2)</i> <i>mutS homolog 6 (MSH6)</i> <i>postmeiotic segregation increased 2 (PMS2)</i>	DNA mismatch repair DNA mismatch repair DNA mismatch repair DNA mismatch repair
Familial adenomatous polyposis	<i>adenomatous polyposis coli (APC)</i>	Wnt signaling
Peutz-Jeghers syndrome	<i>serine/threonine kinase 11 (LKB1/STK11)</i>	Controlling the activity of adenosine monophosphate–activated protein kinase (AMPK) family members
Juvenile polyposis	<i>SMAD family member 4 (SMAD4)</i> <i>bone morphogenetic protein receptor 1A (BMPR1A)</i>	Signal transduction of the transforming growth factor-β (TGF-β) superfamily and bone morphogenetic proteins (BMPs) TGF-β signaling
MYH-associated polyposis	<i>mutY homolog (MUTYH)</i>	DNA base excision repair
Colorectal cancer and familial tooth agenesis	<i>axin 2 (AXIN2)</i>	β-catenin/Wnt signaling
Polymerase proofreading–associated polyposis	<i>polymerase delta 1, catalytic subunit (POLD1)</i> <i>polymerase epsilon, catalytic subunit (POLE)</i>	Catalytic and proofreading subunit of DNA polymerase δ1 Catalytic subunit of DNA polymerase ε

Table 2. Examples of colorectal cancer-associated low-penetrance genetic variants. Such variants can be evaluated in WGS data of a colorectal cancer patient.

Associated chromosomal region	SNP	Candidate affected gene(s)
6p21	rs1321311*	<i>cyclin-dependent kinase inhibitor 1A (CDKN1A)</i>
11q13.4	rs3824999*	<i>polymerase (DNA-directed), delta 3, subunit (POLD3)</i>
Xp22.2	rs5934683*	<i>shroom family member 2 (SHROOM2)</i>
1q41	rs6691170†	<i>dual specificity phosphatase 10 (DUSP10)</i>
3q26.2	rs10936599†	<i>myoneurin (MYNN)</i>
8q23.3	rs16892766†	<i>eukaryotic translation initiation factor 3, subunit H (EIF3H)</i>
8q24.21	rs6983267†	<i>v-myc myelocytomatosis viral oncogene homolog (MYC)</i>
10p14	rs10795668†	(intergenic)
11q23.1	rs3802842†	<i>chromosome 11 open reading frame 93 (C11orf93)</i>
12q13.13	rs11169552†	<i>DIP2 disco-interacting protein 2 homolog B (DIP2B)</i>
		<i>activating transcription factor 1 (ATF1)</i>
14q22.2	rs4444235†	<i>bone morphogenetic protein 4 (BMP4)</i>
15q13.3	rs4779584†	<i>gremlin 1 (GREM1)</i> <i>secretogranin V (SCG5)</i>
16q22.1	rs9929218†	<i>cadherin 1 (CDH1)</i>
18q21.1	rs4939827†	<i>SMAD family member 7 (SMAD7)</i>
19q13.11	rs10411210†	<i>rhophilin, Rho GTPase binding protein 2 (RHPN2)</i>
20p12.3	rs961253†	(intergenic)
20q13.33	rs4925386†	<i>laminin, alpha 5 (LAMA5)</i>

*SNP associations described in (39).

†SNP associations described in (40).

In Iceland, WGS data from 1795 individuals guided researchers to detect a single-nucleotide variant—rs188140481 located at chromosome 8q24—associated with a predisposition to prostate cancer. The strong association between rs188140481 and prostate cancer risk was confirmed in other European populations (11). Relative to variants discovered by genome-wide association studies (GWASs), WGS allowed identification of a rare and novel variant that conferred a disease risk.

POLE (polymerase epsilon, catalytic subunit) and POLD1 (polymerase delta, catalytic subunit)

Mutations in *POLE* and *POLD1*—genes encoding subunits of polymerases ϵ and δ , respectively—were discovered by WGS in the germline of patients with multiple adenomas and/or colorectal cancer (CRC). Focusing on individual families with several affected cases resulted in identification of *POLE* and *POLD1* as high-penetrance CRC susceptibility genes, which were validated with an association analysis in a case-control setting (24).

In an effort combining WGS and exome sequencing, the genetic landscape of 240 high-risk neuroblastoma cases was characterized. In 10 individuals (~4% of the cases), rare candidate pathogenic germline variants were identified in *anaplastic lymphoma receptor tyrosine kinase* (*ALK*), *checkpoint kinase 2* (*CHEK2*), *PTEN induced putative kinase 1* (*PINK1*), *TP53*, *partner and localizer of BRCA2* (*PALB2*), and *BRCA1 associated RING domain 1* (*BARD1*). The variants in *ALK* (one case), *CHEK2* (three cases), and *TP53* (one case) had been previously proposed to be cancer-predisposing; the variants in *PINK1* (two cases), *PALB2* (one case), and *BARD1* (two cases) were novel in this context (25).

These discovery-oriented projects require filtering of the results against adequate controls. This procedure is necessary to identify variants that are present in the cases as well as in the control population, and thus are not likely to be associated with the disease. However, such filtering may remove common variants that play a role in diseases in concert with other genetic and environmental factors. As cohort sizes increase—allowing, for example, stratification of cases and controls in view of exposure—and our analysis pipelines (Fig. 1) improve, the experimental design and ability to detect true causative alleles within individuals and populations also improves. After filtering, many candidate variants remain, and analysis of independent validation sets provides robust statistical proof of association between the variant and the disease. In cases with striking phenotypes, such as multiple primary cancers, WGS is more likely to identify an established or novel cancer predisposition gene. However, for more common and ambiguous phenotypes,

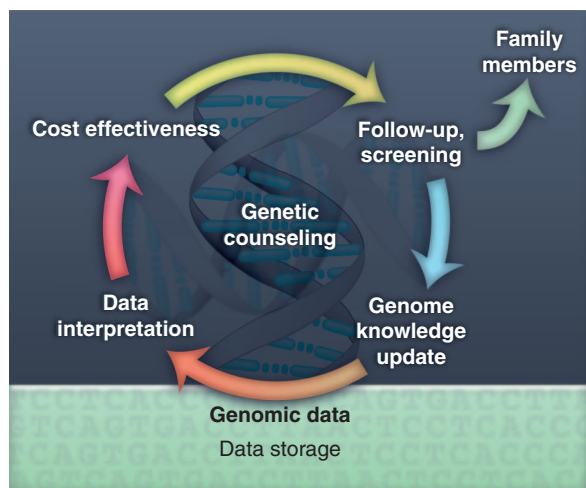


Fig. 2. Cycle of long-term use of WGS data. The full use of WGS data will require regular revisits as new information on data interpretation and management modalities accumulates.

such as breast cancer, the findings are often much less straightforward and can be difficult to interpret (25).

Unambiguous detection of a high-penetrance mutation allows predictive genetic testing in family members followed by lifelong cancer screening in confirmed mutation carriers. The presence or absence of the specific mutation segregating in the family can be tested within the kindred, to identify individuals at risk and those who do not carry the mutation.

Germline Variants Identified by WGS: The Example of Colon Cancer Predisposition

The use of germline data resulting from WGS can help clinicians to focus on the client's genetic susceptibility to a given cancer. We can envision a situation in which a patient has been diagnosed with colon cancer that has metastasized to local lymph nodes. This is a life-threatening condition; however, a good proportion of the patients can be fully cured with surgery and appropriate adjuvant therapies (stage IIIA 5-year survival rate, 73%; stage IIIC 5-year survival rate, 28%; National Cancer Institute SEER database, <http://seer.cancer.gov>). Because one WGS is about as cheap as two colonoscopies (26), tumor DNA and paired normal DNA, after informed consent, may be subjected to WGS for identification of somatic genetic markers that can form a basis for appropriate treatments and follow-up care. With the use of WGS, the patient's germline sequence can be scrutinized for known CRC predisposition loci; heritable factors play a role in one-third of CRC cases (27), with about 5% of CRCs displaying dominant or recessive Mendelian inheritance.

High-Penetrance Variants

Hereditary cancer predisposition syndrome is suspected when there are (often unspecific) signs

such as young age at disease onset and multiple primary tumors (28). Because modern families are typically small and may lack a family history of cancer, it is not surprising that most cases with CRC predisposition cannot be unambiguously diagnosed on the basis of clinical features and family history.

The most common familial CRC syndrome is Lynch syndrome, found in approximately 3% of CRC patients (20) and also known as hereditary nonpolyposis colorectal cancer (HNPCC). Lynch syndrome is caused by an autosomal dominant mutation in DNA mismatch repair (MMR) genes [*mutS homolog 2* (*MSH2*), *mutS homolog 6* (*MSH6*), *mutL homolog 1* (*MLH1*), or *postmeiotic segregation increased 2* (*PMS2*); reviewed in (29)]. Individuals carrying these mutations have a more than 50% lifetime risk of developing CRC, but also have an increased risk for endometrial cancer and other intra-

abdominal adenocarcinomas (30). More rare familial CRC syndromes include familial adenomatous polyposis, Peutz-Jeghers syndrome, juvenile polyposis, MYH-associated polyposis, and polymerase proofreading-associated polyposis (24) (Table 1). Appropriate surgical treatment and follow-up for individuals with high-penetrance CRC syndromes is usually available. In particular, Lynch syndrome lends itself well to early intervention, as regular colonoscopy screenings reduce mutation carriers' mortality and morbidity (31). Drug-based CRC prevention modalities are also emerging for Lynch syndrome (32), and clinical genetics units now ensure that counseling and screening of mutation-positive individuals and their families are provided.

Germline genetic tests extend past other medical tests in that genetic test results reach beyond the individual in question, in cases where the WGS information leads to a need for clinical intervention such as lifelong follow-up for cancer predisposition. Further complicating matters is the consideration of whether close relatives have the right and/or desire to know the relevant results. Finding a high-penetrance CRC-predisposing mutation in an index patient warrants counseling and possible testing for the mutation in the patient's first-degree relatives. Although such genetic testing and cancer prevention procedures are, in principle, established in many countries, often health care systems are too stretched to achieve high compliance (33).

Low-Penetrance Variants

Beyond individuals with Lynch syndrome, GWASs of CRC cases and population-based matched controls have identified specific SNPs that are more common in CRC cases than in the general population (Table 2). Most of these polymorphisms lack any known function but may be surrogate markers for linked causative genetic variants. One causative variant, SNP rs6983267 at 8q24, appears

to be directly involved in regulation of the onco-gene *v-myc myelocytomatosis viral oncogene homolog (MYC)* (34), where the risk allele appears to increase activity of an enhancer element.

A functional basis has been elucidated for three other association signals as well, namely *eukaryotic translation initiation factor 3, subunit H (EIF3H)* at 8q23.3, *bone morphogenetic protein 4 (BMP4)* at 14q22.2, and *SMAD family member 7 (SMAD7)* at 18q21.1 (35–37). Although these risk variants are common—for example, most humans carry one or two rs6983267 risk alleles—they confer only a slightly higher risk for CRC relative to individuals carrying alleles not linked to cancer and assumed to be functionally neutral. In CRC, 20 genomic loci have been identified through GWASs as markers for CRC risk (38–40). The presence of each of these SNPs confers only a very slightly increased risk [odds ratio (OR) = 1.07 to 1.28]. Additional variants with similar risk have emerged from studies of high-penetrance CRC predisposition genes. These variants include *adenomatous polyposis coli (APC)* I1307K (41) and SNP rs1800734, which maps to the *MLH1* gene promoter (42).

Genetic factors, however, function in concert, and their cumulative effect on risk may be higher. Carrying 19 or more of the 28 possible CRC-associated alleles increases the risk of CRC by a factor of 2.3 (40). Although the presence or absence of a risk allele is easy to score from the WGS data, at present this information is of little clinical importance. This may change with a more exact view of the risk factors. Identifying all risk factors requires extensive studies on cohorts with genetic, exposure, and lifestyle information, as well as functional studies examining the molecular mechanisms involved (43).

Variants of Uncertain Significance

Variants of uncertain significance (VUSs)—changes in the sequence that are impossible to interpret with current knowledge—pose a major challenge in tests that examine multiple high-penetrance cancer predisposition genes. VUSs have been extensively studied in the context of *BRCA1* and *BRCA2* mutation screenings; 30% of tests reveal at least one VUS, and ongoing efforts seek to improve the classification of such variants from in silico as well as clinicopathological information (44). Similarly, many individuals carry VUSs in high-penetrance CRC genes (45, 46). CRC-predisposing genes are relatively well characterized for identified causative mutations, and novel truncating variants can often—although not always—be interpreted as pathogenic (47). However, in the many genes to be considered for hereditary CRC susceptibility alone, situations will arise where missense-type variants of unknown importance are detected. Functional evaluation of such variants is usually not available for diagnostic purposes, resulting in uncertainty. In practice, identification of VUSs results in a test providing little useful information. The possibility of such an outcome should be explained to the client be-

fore testing. As most medical professionals have limited backgrounds in medical genetics (48, 49), the frequent occurrence of VUSs may hinder the clinical implementation of genetic screening.

Practical Considerations for the Use of WGS Germline Data

The complexities involved in using WGS to analyze cancer require careful advance consideration of how much information extracted from the effort should be passed to the client. Because of our incomplete knowledge of the function of the human genome, communicating the results of an open-ended WGS test to clients may be challenging. Although more appropriately qualified experts are needed, it is equally important to educate nonspecialist health care professionals and provide them with established procedures to follow, to enable them to cope with the tasks ahead. Adding to the challenges in counseling is the fact that clients are increasingly individuals from the general population, varying in education and genetic expertise, who exhibit cancer and other serious conditions. Ideally, the level of communicating the WGS analysis results should be tailored case by case on the basis of patients' expressed desires after informed consent. For some individuals, it might be feasible to provide results only for variants that are useful to know and are easy to interpret and explain, such as certain genetic variants associated with drug metabolism (14) or with severe but preventable disease; other patients may request additional information gleaned from the analysis of their WGS. Opportunities to revisit the data to counsel and answer questions at a later date should be available and would allow use of the WGS information in a structured manner (Fig. 2). Adoption of guidelines for a restricted approach to communicating data would also protect the genetic privacy of children and adolescents undergoing WGS (50).

Future Prospects

The ultimate goal of WGS is to make predictions from the genetic composition of the individual. Comprehensive understanding of the WGS data needs to take into account that the phenotypes arising from any specific genotype are context-dependent and work in concert with environmental stimuli (43). Progress toward this goal will result in personalized risk assessment, specifically the identification of cancer risks (due to mono- or polygenic inheritance or low tolerance for environmental exposures) before tumor development. In the case of modest risk profiles, it is difficult to predict how eagerly individuals will adopt personalized cancer prevention strategies, which will likely be similar to those suggested without the benefit of WGS.

The availability of WGS is a great achievement for humankind. However, we still need international coordination in WGS implementation, in particular to improve interpretation and to design cost-effective and ethical management strategies.

National approaches for implementation, matching the local resources, must be developed to make the best use of WGS's potential in disease prevention.

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REVIEW

Cancer Pharmacogenomics: Early Promise, But Concerted Effort Needed

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The past decade has brought together substantial advances in human genome analysis and a maturation of understanding of tumor biology. Although there is much progress still to be made, there are now several prominent examples in which tumor-associated somatic mutations have been used to identify cellular signaling pathways in tumors. This in turn has led to the development of targeted therapies, with somatic mutations serving as genomic predictors of tumor response and providing new leads for drug development. There is also a realization that germline DNA variants can help optimize cancer drug dosing and predict the susceptibility of patients to the adverse side effects of these drugs—knowledge that ultimately can be used to improve the benefit:risk ratio of cancer treatment for individual patients.

Mechanistic understanding of the biologic pathways regulating human cancers and the normal cells from which they are derived has long influenced the management of cancer. These efforts have shifted from older, cytotoxic therapeutic options toward chemical and biologic therapies that are precisely designed to target a critical gene or pathway. This has delivered a degree of tumor control for common cancers, including breast, lung, and colorectal, and extended life and provided cures in some cases of less common cancers, such as testicular cancer and childhood acute lymphoblastic leukemia. Pathway-driven therapeutics has substantially improved the outcomes of chronic myelogenous leukemia and gastrointestinal stromal tumors which may, in the absence of relapse, act as chronic diseases requiring life-long treatment, akin to diabetes or hypertension management (*1*). However, these advances have come at a cost, both literally and figuratively, with newer treatments often costing thousands of dollars per month and associated with toxicities that can negatively affect patient quality of life.

Somatic mutations, variations found within the tumor, and germline mutations, heritable variations found within the individual, may influence disease outcome and/or response to therapy (Fig. 1). These mutations, or cancer biomarkers, can be broadly classified as prognostic markers—those mainly associated with the course or outcome of a disease—or predictive markers, which can be used to identify subpopulations of patients who are most likely to respond to a given therapy. There is opportunity for genetic informa-

tion to aid both the selection of effective therapy and the avoidance of treatments with an unacceptable risk of adverse drug reactions.

Inherited differences in drug effects were first documented in terms of drug metabolism in the 1950s (*2, 3*), giving rise to the term “pharmacogenetics.” The field has now extended to all aspects of drug disposition, including absorption, distribution, and excretion (*4*), as well as drug tar-

gets and downstream effect mediators. Table 1 outlines some current examples where genotype is used for the selection of cancer chemotherapy.

Tumor Profiling: From Discovery Science to Patient Management

Analysis of tumor DNA to guide patient treatment has been used for more than 20 years. An acute lymphoblastic leukemia patient with the presence of a 9:22 chromosomal translocation was once offered bone marrow transplantation, rather than standard cytotoxic chemotherapy; more recently, these patients would be offered imatinib or other *ABL* tyrosine kinase inhibitors. A breast cancer patient with amplification of *HER2* might be treated with the anti-*HER2* monoclonal antibody trastuzumab or the *HER2* tyrosine kinase inhibitor lapatinib. Thus, focused profiling is becoming part of routine patient management for select cancers (Table 1) as the lowered costs of high-quality DNA sequencing have led to the identification that some somatic mutations are associated with specific benefits (or lack thereof) from targeted therapies.

Somatic DNA mutation assessment has positively impacted patient care for a limited number of cancers. The identification of *KRAS* mutations in codons 12 or 13 in ~30% of patients with colon cancer suggests that there is no tumor control

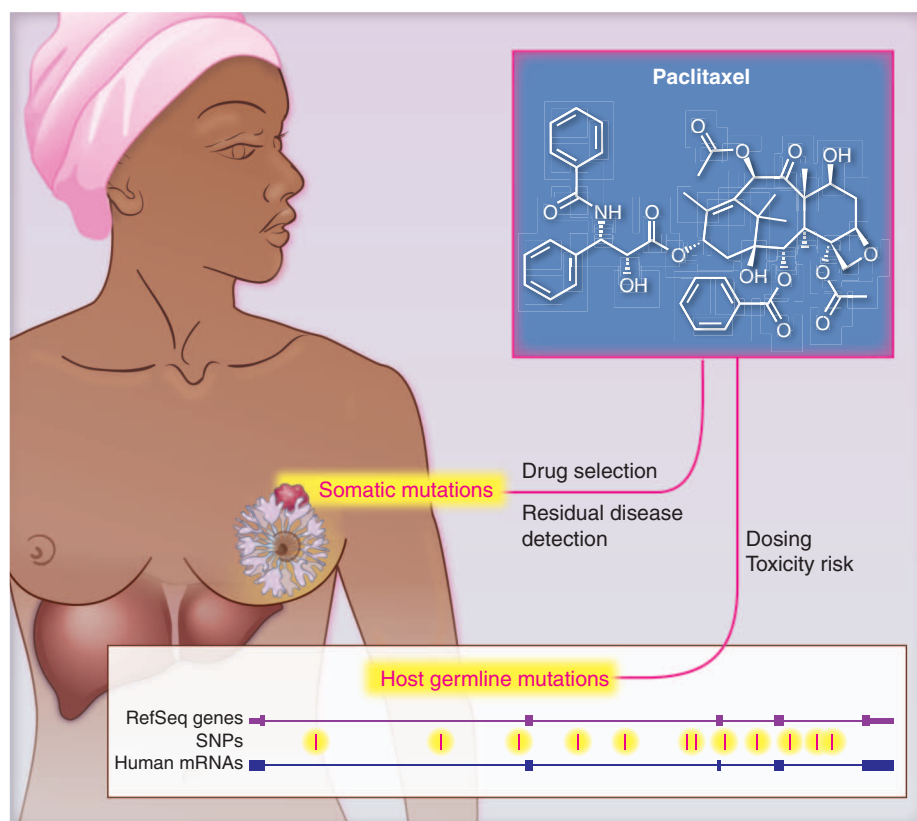


Fig. 1. Attention must be paid to both tumor and host. Cancer pharmacogenomic variation in both the tumor (somatic changes) and normal tissues (germline variants) influence the treatment of cancer patients.

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benefit, but some toxicity risk, when patients are treated with expensive antibodies targeting epidermal growth factor receptor (EGFR) (5). Lung cancer, melanoma, and myeloproliferative disorders tend to be sensitive to tyrosine kinase inhibitors with mutations in the respective genes *EGFR*, *BRAF*, and *JAK2*. However, currently there are molecular predictors of efficacy for less than 10% of the cancer drugs approved by the U.S. Food and Drug Administration (FDA).

In addition, cancer cells may mutate and evolve resistance to specific drug treatments, resulting in the proliferation of drug-resistant tumors. Beyond the initial patient treatment setting, there is a lack of personalized cancer medicine trial data on which to guide patient management decisions. Treatment choices often revert to the use of population average data, where it is difficult to ascertain the value of therapy for an individual patient. We still need definitive discovery and validation/replication efforts for anticancer

drugs (old and new). This is particularly true for the older cytotoxic agents, which benefit a meaningful subset of patients, but do not have the diverse scientific and financial advocacy to assure that genomic knowledge is being discovered and deployed in a clinically relevant manner.

As sequencing strategies mature and costs are lowered, there has been an increase in the application of these technologies to tumor profiling (6, 7). Although the current focus is principally directed toward the identification of somatic DNA mutations, cancer may be associated with epigenetic traits, including specific microRNAs, variations in RNA expression, methylation patterns, and chromatin marks. Currently, the most common genetic screening involves performing a targeted DNA capture, focused on a few relevant candidate genes, followed by sequencing (8). This gives a clinical report that may direct treatment to a signaling pathway that would not be of obvious importance from tumor histology.

Genomic medicine strategies that can identify and clinically annotate the broad assortment of actionable variants are needed to justify these efforts. An initial deep sequencing of 145 genes in colorectal and non-small cell lung cancers found somatic mutations in 39 of 40 (98%) and 20 of 24 (83%) tumors, respectively (8). More than half (52.5%) of colorectal cancers and 72% of non-small cell lung cancers contained at least one mutation that has been linked to a specific chemotherapy approach (8). Similar data have come from the National Cancer Institute–National Human Genome Research Institute (NCI-NHGRI) Cancer Genome Atlas efforts across tumors from diverse anatomical locations (6, 7).

Clinical pharmacogenomic efforts to apply deep sequencing to unveil mechanisms of sensitivity or resistance to drug therapy are needed, as we do not know the mechanism of clinical resistance for most anticancer drugs. Sequencing of non-small cell lung cancer that displayed sensitivity and subsequent resistance to EGFR tyrosine kinase inhibitors led to the routine use of *EGFR* sequencing to guide therapeutic choices (9). Whole-exome sequencing of patients treated with everolimus for advanced bladder cancer revealed that a specific *TSC1* mutation correlated with everolimus sensitivity. Patients with *TSC1* mutation had a longer time until recurrence of tumor (4.1 versus 1.8 months) (11). This loss-of-function mutation in *TSC1* was subsequently found in 5 of 96 (5.2%) advanced bladder cancers, suggesting that there is a subgroup of patients with this disease for whom everolimus treatment might offer substantial benefit.

There are limits to how much tissue can be acquired from a clinical biopsy. The practical issue of low availability of high-quality tumor DNA is helping drive analysis from single-gene assays to multigene applications, where more knowledge is derived from the existing tissue. Also, quality control issues, resulting in uncertain or erroneous identification of mutations from the use of gene panels or whole-genome assessment, may challenge interpretations of molecular diagnostic results across clinical laboratories. Furthermore, we need predictive analyses for the 25 to 80% of cases where variants of unknown significance are identified in genes that are of interest to a particular tumor. This has been evident in *BRCA1/2* testing, associated with breast cancers. We need to generate both laboratory and clinical consensus methods to decide which variants in which genes merit clinical action (10).

For certain tumor types, clinical trial inclusion criteria are starting to focus less on the anatomical origins and more on the somatic mutations identified within a tumor. The focus on “driver” mutations controlling tumor invasiveness and its relative therapeutic response requires screening many patients to find the few that are eligible for a targeted therapy trial (11). This is not easily implemented at academic centers as currently a

Table 1. Pharmacogenomic DNA markers in clinical use for chemotherapy or supportive care of cancer patients.

Germ line	Somatic	Drugs	Effect
Thiopurine methyltransferase	—	Mercaptopurine, thioguanine	Neutropenia risk
UDP-glucuronosyltransferase 1A1	—	Irinotecan, nilotinib	Neutropenia risk; underdosing risk
Glucose-6-phosphate dehydrogenase	—	Rasburicase	Anemia
Cytochrome P450 2D6	—	Codeine, oxycodone; tamoxifen	Altered pain control; altered drug dose
—	Janus kinase 2 (JAK2)	Ruxolitinib	Altered drug activity
—	Human epidermal growth factor receptor 1 (EGFR)	Cetuximab Erlotinib Gefitinib	Altered drug activity
—	Kirsten rat sarcoma viral oncogene homolog (KRAS)	Panitumumab Cetuximab	Lack of drug activity
—	Abelson murine leukemia viral oncogene homolog 1 (ABL)	Imatinib, dasatinib, nilotinib	Altered drug activity
—	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT)	Imatinib	Altered drug activity
—	Human epidermal growth factor receptor 2 (HER2)	Lapatinib Trastuzumab	Enhanced drug activity
—	v-Raf murine sarcoma viral oncogene homolog B1 (BRAF)	Vemurafenib	Enhanced drug activity
—	Anaplastic lymphoma receptor tyrosine kinase (ALK)	Crizotinib	Altered drug activity

disproportionate load of patients are referred for experimental therapy without a previously ascertained molecular profile. This introduces additional time and unsupported expense for somatic sequencing before a transition into a treatment trial of relevance.

There are also unanticipated issues, such as the current preference for fresh tissue, requiring a tissue biopsy for the purposes of the somatic profile. Many clinical practice settings do not have ready access to interventional radiologists for safe biopsy of tumor, nor do they have personnel trained to properly handle tissue to best retain a tumor's molecular signature (most tissues from community oncologists are placed in a formalin-containing fixative and sent to an outside pathologist). Sequencing efforts targeted at formalin-fixed, paraffin-embedded tissue and nucleic acid detection in plasma or identification of circulating tumor cells may also provide a means to circumvent some of these barriers. Well-designed practical infrastructure is needed for the application of personalized cancer medicine to ensure that all the right team members will be trained and ready to provide patient support. Commercial and academic efforts that focus on disease-specific gene targets, such as the National Comprehensive Cancer Network or Foundation Medicine, are actively developing the pathways for application in patient management (12). As the costs decrease and the interpretation ability increases, somatic DNA assessment will become a routine part of the management of cancer.

The Role of Germline DNA in Optimizing Dosing and Identifying Toxicity Risk of Anticancer Drugs

Although the primary focus of cancer genomic research is on the somatically mutated genes driving tumor growth, interpatient germline variation can also impact cancer treatment. Indeed, given that oncology supportive care is mostly targeted toward mollifying a patient's adverse effects of cancer treatment while eradicating the cancer, genetic variation can potentially play an important role in the selection and administration of cancer drugs.

There is also an undervalued role of gastrointestinal drug transport and hepatic drug metabolism on the dose, administration schedule, and route of administration of a drug. The use of a highly targeted, effective therapy for chronic myelogenous leukemia can be undone by underdosing, if the drug is metabolized or removed before it encounters the intended cell or molecular target, setting up a milieu for development of drug resistance (13). Pharmacogenomic variation in drug metabolism has been shown to have a role in the efficacy of certain anticancer therapies such as tamoxifen, an effective antiestrogen used in the treatment of hormone receptor-positive breast cancer. Bioconversion of tamoxifen to several active metabolites including endoxifen, its most abundant active metabolite, is primarily de-

pendent on the highly polymorphic cytochrome P450 2D6 (CYP2D6) enzyme. In the past, tamoxifen was often coadministered with certain antidepressants for treatment of antiestrogen-induced hot flashes until it was discovered that the antidepressants blocked CYP2D6 and the production of endoxifen (14). Coadministration was abruptly discontinued in clinical practice, because of the risk that the "cure" of hot flashes was compromising the effectiveness of the antiestrogen therapy (15). Clinical trials of endoxifen as a treatment for breast cancer are now under way (16).

In addition, ~7% of the U.S. population have a genetic polymorphism or deletion in CYP2D6, resulting in diminished protein abundance and/or function. More than 20 published studies have reported an association between CYP2D6 polymorphisms and breast cancer outcomes after tamoxifen treatment, although several recent studies suggest that homozygote variant patients that have no or lower function of CYP2D6 have the poorest outcome (17). Studies to determine the appropriate dose of tamoxifen for wild-type patients (called extensive metabolizers), and heterozygous patients (intermediate metabolizers), suggest that doubling of tamoxifen dose in intermediate metabolizers normalized plasma endoxifen concentrations to that observed in extensive metabolizers (18, 19). This level of data is consistent with that usually required for FDA-prescribing recommendations for dose adjustment after organ dysfunction, drug interaction, or age and suggests a relevance to CYP2D6-guided tamoxifen dosing as a routine part of reducing both interpatient variation and the risk of underdosing patients with breast cancer.

In most discussions of clinical management of cancer patients, the risk/benefit assessment focuses on the probability of tumor control from a specific drug, in part because there is a deficiency of objective data with which to assess the patients' risk of developing severe adverse drug effects. This deficiency has prompted the launch of genomic discovery programs that focus on adverse effects of cancer drugs such as sensory peripheral neuropathy, cardiotoxicity, hearing loss, and other toxicities (2, 20–23). For example, the microtubule inhibitor paclitaxel is used to treat breast, lung, and ovarian cancers, but paclitaxel-induced neuropathy is a common adverse event that often leads to therapeutic disruption and patient discomfort (24). However, there are currently no mechanisms for prospective identification of patients at heightened risk for neuropathy, for whom choices of drug, administration schedule, and quality of life could be informed.

Both candidate gene and genome-wide association studies (GWAS) have begun to inform the prediction of patient risk for neuropathy. A recent GWAS involved 855 subjects of European ancestry where paclitaxel was administered as part of therapy for lymph node-negative breast cancer. This study identified a single-nucleotide polymorphism in the *FGD4* gene, which encodes FGD1-

related F-actin binding protein, that was associated with the onset of sensory peripheral neuropathy in the discovery cohort (hazard ratio = 1.57; 95% confidence interval 1.30–1.91) and was also observed in a European and African-American replication cohort (20). As *FGD4* is a congenital peripheral neuropathy gene, there is biologic plausibility to further assess the contribution of genetic variation to the development of peripheral neuropathy.

Another study identified a near-doubling of increased risk of paclitaxel-induced neuropathy related to CYP2C8*3 status in breast cancer patients (21). Although CYP2C8*3 is less common in African-Americans, a significant association was replicated in direction and magnitude of effect. The observation of increased risk of paclitaxel-induced neuropathy in patients who carry the CYP2C8*3 variant across racially distinct patient cohorts suggests that both pharmacokinetic variables (such as CYP2C8) and biologic variation (such as *FGD4*) contribute to patient risk of neuropathy.

The avoidance of chemotherapy-associated morbidity is critical in the context of adjuvant chemotherapy, where the goal is to kill any stray cancer cells that might have gone undetected. This is especially important in the treatment of childhood malignancies, as many patients will survive their cancer and experience the sequelae from adverse drug events for many years. Cardiomyopathy from anthracycline chemotherapy is a devastating morbidity, with long-term effects on patient productivity and quality of life. Germline variation associated with risk of cardiomyopathy has been identified by means of distinct candidate gene strategies and different patient recruitment strategies. Similar data on germline pharmacogenomics predictors of cisplatin-associated damage to hearing ototoxicity has also been reported, providing a mechanism for prospective identification of patients at risk for this debilitating morbidity (22).

There are major limitations to pharmacogenomics discovery for anticancer therapies, including the challenges of building large patient cohorts for both discovery and validation purposes. It often takes 7 to 10 years to construct, conduct, and analyze a clinical trial, which can then be used for pharmacogenomics discovery. The same is true for a validation cohort, which is one reason that there are so few discovery and replication studies in the literature. There is also a paucity of information on the heritability of anticancer drug effects, to help justify the quest for genomic solutions to variability in drug effect. One approach is the recent use of cell lines from large, multigenerational families, which have shown a wide variation in heritability of cytotoxicity (10 to 70%) across 29 commonly prescribed anticancer drugs, with 66% having greater than 30% heritability (25). This presents an opportunity for both prioritization of drugs for assessment and conduct of ex vivo discovery that will allow precious clinical material to be used for validation studies. The application of bar coding and robotics

has allowed the scale-up of cell line phenotyping to 500 to 1000 cell lines per project, followed by ex vivo GWAS (25–27). Innovative pharmacogenomics strategies will allow us to more rapidly capture the relevance of genomic information for rational drug therapy selection (Fig. 2).

Moving into Clinical Practice

Both replication and validation of pharmacogenomics traits raise challenges. It is often difficult to characterize, uniformly treat, and systematically evaluate patients to objectively quantify the drug response phenotype. The standard of care should be to obtain genomic DNA from all patients entered into clinical drug trials, along with appropriate consent to permit pharmacogenetic studies. This is now accomplished in most large trials being conducted by pharmaceutical companies and is routine for some of the NCI clinical trials groups (20, 28, 29), but has not yet become standard for academic or foundation-supported trials (Fig. 2).

The challenge is to balance the desire to apply new information and the need to ensure that there are robust data supporting the idea that acting on a pharmacogenomic marker is in the best interest of the patient. The reliance on prospective, randomized, controlled trials as the only way to justify clinical implementation is not practical and guarantees that new information will have a 5- to 10-year lag while studies are constructed, conducted, and interpreted. There is also a disconnection between the funding bodies and the prioritization of this type of study, in terms of financial commitment, clinical trial infrastructure, and ability to rapidly enact new strategies. There have been several efforts to develop ways to gain confidence in early adoption of pharmacogenomics data, on the basis of consensus building among institutions around the applica-

tion of genetic information to drug therapy. One such effort is the Clinical Pharmacogenetics Implementation Consortium (CPIC), which includes participants from >80 institutions across four continents (30). A key element to programs such as CPIC is the realization that there are some aspects of the medical decision process, such as drug dosing, that have robust data to benefit patients, even as the field waits for the “perfect” studies that definitively guide therapy at a broader level.

Double standards still exist in clinical decision-making, in that a drug interaction may be accepted as a credentialed, clinically relevant variable and rapidly integrated into clinical practice, yet the application of genetic data through the same mechanism is delayed owing to the need to accumulate large amounts of prospective data. This is occurring for CYP2D6 and tamoxifen, CYP3A4 and taxane chemotherapy, and related interactions for supportive care medications. The standard for drug interaction is influenced by years of familiarity and no additional time or expense to the patient, but has less functional predictability than gene deletion in the same pathway. There is a need to devise a framework whereby any source of variation in a clinically credentialed pathway can be moved toward clinical implementation.

The endpoints of pharmacogenomics studies have followed a traditional biomarker scheme, trying to explain untoward events, identify low utility, define dose selection, or preemptively predict severe drug reactions. These are important endpoints and should not be neglected in investigational endeavors. However, there are alternative endpoints that are typically considered too mundane for inclusion in NIH grants, but are major drivers of early adoption for new health care modalities. These include avoidance of 30-day readmission rates, economics of “bundled care,”

and the prioritization of medication access by a health system Pharmacy and Therapeutics committee. These endpoints are often accessible through observational cohorts or electronic health record studies and will likely drive the implementation of pharmacogenomics into practice.

It is time to be more practical as we move forward. Although substantial progress has been made in identifying and characterizing pharmacogenomic phenomena, translation of these data into practical clinical applications remains slow. A variety of factors contribute to this problem, including a lack of clarity on the amount of data needed to prove clinical utility, the paucity of interventional pharmacogenetic studies, and unresolved practical considerations, such as how to establish and implement clear guidelines in departments that manage cancer. There are also societal factors at play, including acceptance of widespread genetic testing as well as implications for insurance coverage and liability. These issues will need to be explored and addressed before the promise of genetically customized medicine can become a reality.

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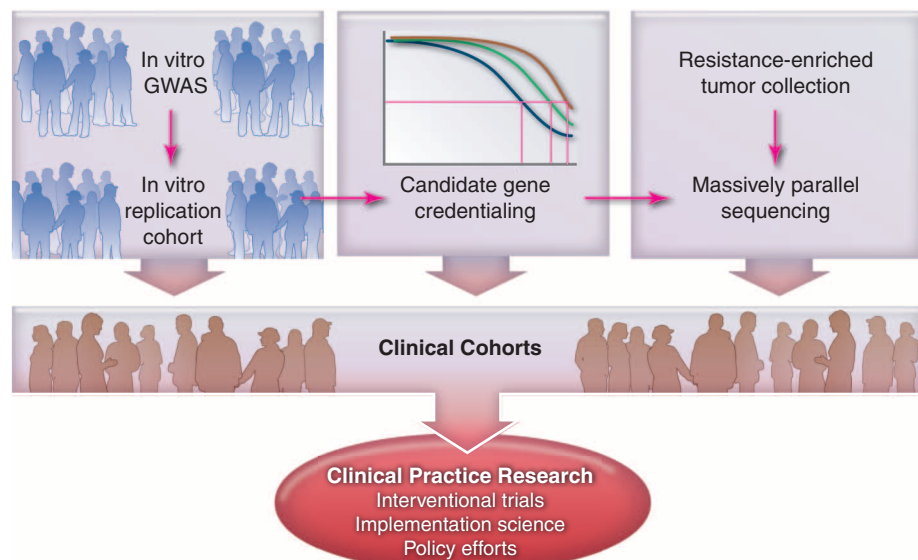


Fig. 2. A multidimensional strategy is required. A blend of in vitro, ex vivo, and in vivo strategies are needed to more rapidly move the promise of pharmacogenomics into application.

REVIEW

Epigenetic Reprogramming in Cancer

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The demonstration of induced pluripotency and direct lineage conversion has led to remarkable insights regarding the roles of transcription factors and chromatin regulators in mediating cell state transitions. Beyond its considerable implications for regenerative medicine, this body of work is highly relevant to multiple stages of oncogenesis, from the initial cellular transformation to the hierarchical organization of established malignancies. Here, we review conceptual parallels between the respective biological phenomena, highlighting important interrelationships among transcription factors, chromatin regulators, and preexisting epigenetic states. The shared mechanisms provide insights into oncogenic transformation, tumor heterogeneity, and cancer stem cell models.

Specification of cellular fate during development is a dynamic process by which diverse phenotypes are established in precise

temporal and positional patterns. Beginning from a single totipotent cell, successive waves of self-renewal, differentiation, and commitment ultimately yield the intricate array of cell types, tissues, and organs of a fully formed organism. DNA sequence-specific transcription factors (TFs) play a prominent role in fate specification, as demonstrated by seminal studies of the muscle fate master regulator MyoD (1) and the core TFs that mediate pluripotency (2, 3). The classical dogma by which TFs act within proximal promoters to

initiate transcription has been expanded by the identification of staggering numbers of distal enhancer-like elements in the human genome, which are activated by TFs in combinatorial and highly cell type-specific patterns (4, 5).

To exert their proximal and distal regulatory activities, TFs must contend with the underlying organization of chromatin, a higher-order structure of DNA, RNA, histones, and regulatory proteins (6, 7). TFs recruit chromatin regulators (CRs) that modulate the accessibility of target DNA and impart specific chromatin states characterized by signature histone modifications and common functional roles (4). However, because TF binding is dependent on chromatin accessibility, CRs and chromatin states also function as gatekeepers that modulate TF regulatory activities. Differentiation events frequently rely on promoters and enhancers that are “poised” by pioneer TFs and characteristic chromatin configurations (4, 8). Thus, a hierarchy of TFs, cooperating CRs, and coordinated chromatin states guide successive differentiation and commitment events during developmental specification (Fig. 1).

Lessons from Induced Pluripotency

In 2006, Shinya Yamanaka demonstrated induced pluripotency, whereby a differentiated cell can be

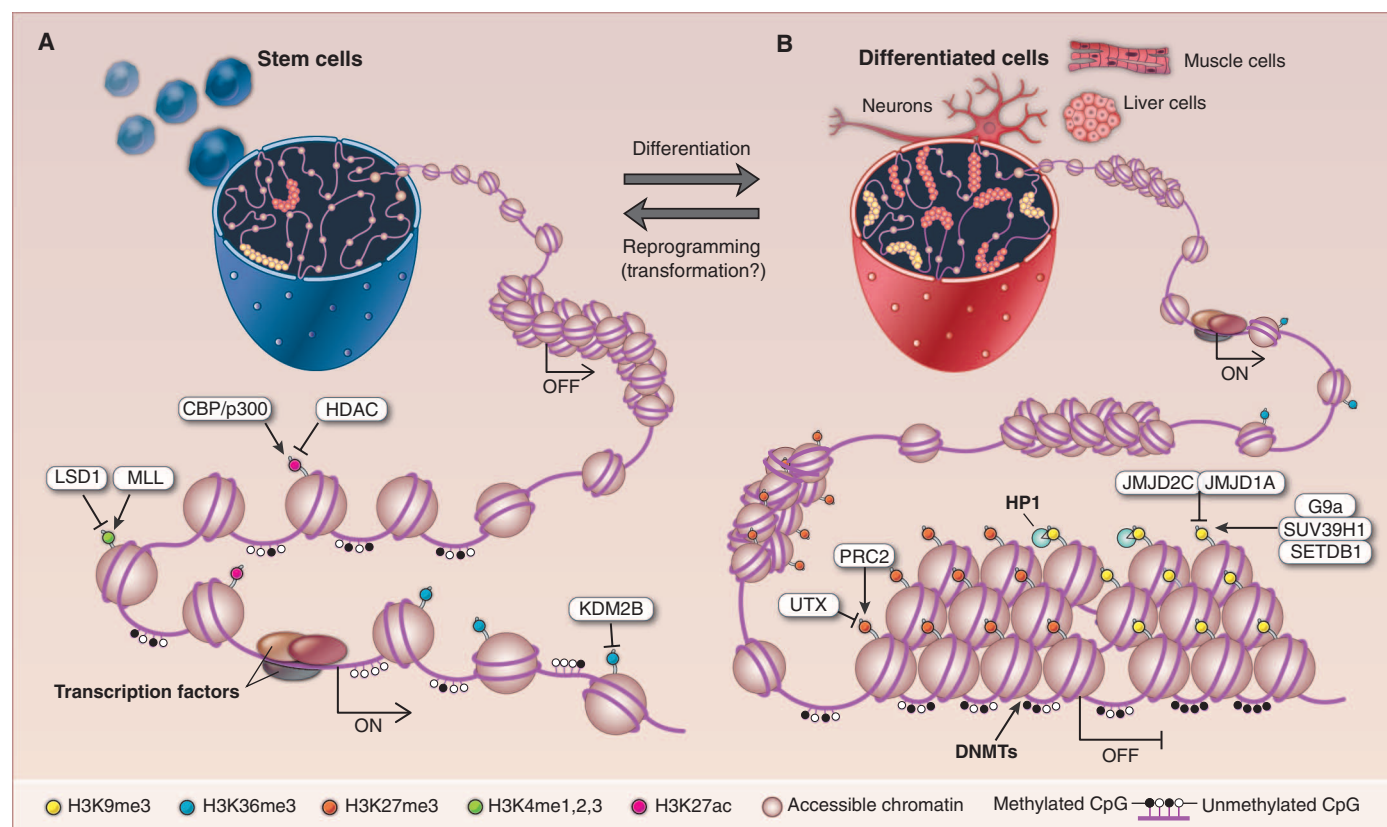


Fig. 1. Developmental specification is associated with global alterations in chromatin structure. (A) In pluripotent cells, chromatin is hyperdynamic and globally accessible. (B) Upon differentiation, inactive genomic regions may be sequestered by repressive chromatin enriched for characteristic histone modifications

and refractory to regulatory activity. These global structures are regulated by DNA methylation, histone modifications, and numerous CRs whose expression levels are dynamically regulated through development. In addition, transcriptional changes are accompanied by focal alterations in chromatin structure at specific gene loci.

directly reprogrammed into an induced pluripotent (iPS) cell by a defined set of TFs (2, 3). The Nobel Prize-winning discovery represented a seminal advance for the fields of stem cell and regenerative biology. Yet the finding and a flurry of follow-up studies may have equally profound implications for cancer biology. The body of work demonstrates the dramatic consequence of deploying gene regulatory mechanisms in inappropriate developmental contexts. It provides key insights into the mechanisms of action of TFs, CRs, and chromatin states that direct, facilitate, or hinder cell fate transitions. A striking number of the implicated factors and mechanisms are now recognized to play critical roles in malignant transformation. Here, we draw upon these shared themes in an examination of genetic and epigenetic mechanisms that contribute to cellular reprogramming and cancer.

Induced pluripotency was initially demonstrated by reprogramming fibroblasts with four TFs: Oct-4, Sox2, Klf4, and c-Myc. Only the core factors, Oct-4 and Sox2, are strictly required, whereas the other components may primarily enhance reprogramming efficiency and can be substituted by other genes such as Nanog and Lin28 (2, 3, 9). Demonstrations of direct conversion between cell lineages reinforce that master TFs determine cellular identity (10, 11). The right combination of TFs can drive state transitions, binding synergistically to promoters and enhancers to activate gene networks. Reprogramming also involves focal and

global changes to chromatin structure as required to reset the epigenetic landscape (12). In iPS reprogramming, de novo chromatin activation, mediated by TF recruitment of CRs and associated transcriptional changes, occurs early (13). In contrast, the formation of bivalent domains and the global chromatin decondensation characteristic of pluripotent cells appear to represent later events (3, 12). These changes involve chromatin modification and remodeling, rendering reprogramming dependent on CRs that catalyze these activities. Moreover, preexisting chromatin states and DNA methylation can present roadblocks that impede TF binding and gene induction, thus hindering cell state transitions (14–16).

Reprogramming and Cancer Epigenetics

Oncogenic transformation frequently involves de novo acquisition of developmental programs, analogous to cellular reprogramming, and yields cells with unlimited self-renewal potential, a feature shared with iPS and other directly reprogrammed stem cells. This parallelism is supported at a mechanistic level by facilitators and barriers shared between the processes (12). Several reprogramming TFs represent bona fide oncogenes, whereas many genes that act as barriers to reprogramming correspond to known tumor suppressors, including p53 and Ink4A/Arf, whose effects on proliferation and apoptosis impede both processes (12). Similarly, CRs play essential roles as effectors

and modulators of reprogramming and have established functions in oncogenesis (7, 12). These general principles suggest that epigenetic rewiring necessary for cellular reprogramming may be recapitulated in part during cellular transformation.

Transcription Factors

Each of the iPS reprogramming factors has established roles in oncogenesis (Fig. 2A). Oct-4 plays a driving role in initiating germ cell tumors and represents an important diagnostic marker (17). Sox2 is amplified in squamous cell carcinoma of the lung and esophagus (18) and small-cell lung carcinomas (19), acting as a lineage-survival oncogene in each of these tumors. Sox2 is also an essential driver of cancer stem cell subpopulations in glioblastoma, breast cancer, and Ewing sarcoma, consistent with its vital role in pluripotent and tissue stem cells (Fig. 2A) (20, 21).

c-Myc functions in a wide range of human malignancies. Its expression may also explain the tendency of mice generated from iPS clones to spontaneously develop tumors (2). c-Myc may drive proliferation through induction of common gene expression programs in embryonic stem (ES) cells and various malignancies. Alternatively, c-Myc may function as a global amplifier of gene expression through its potent effect on the RNA polymerase II elongation factor P-TEFb (22, 23). This alternative view may explain how a single oncogene can reinforce the pluripotency

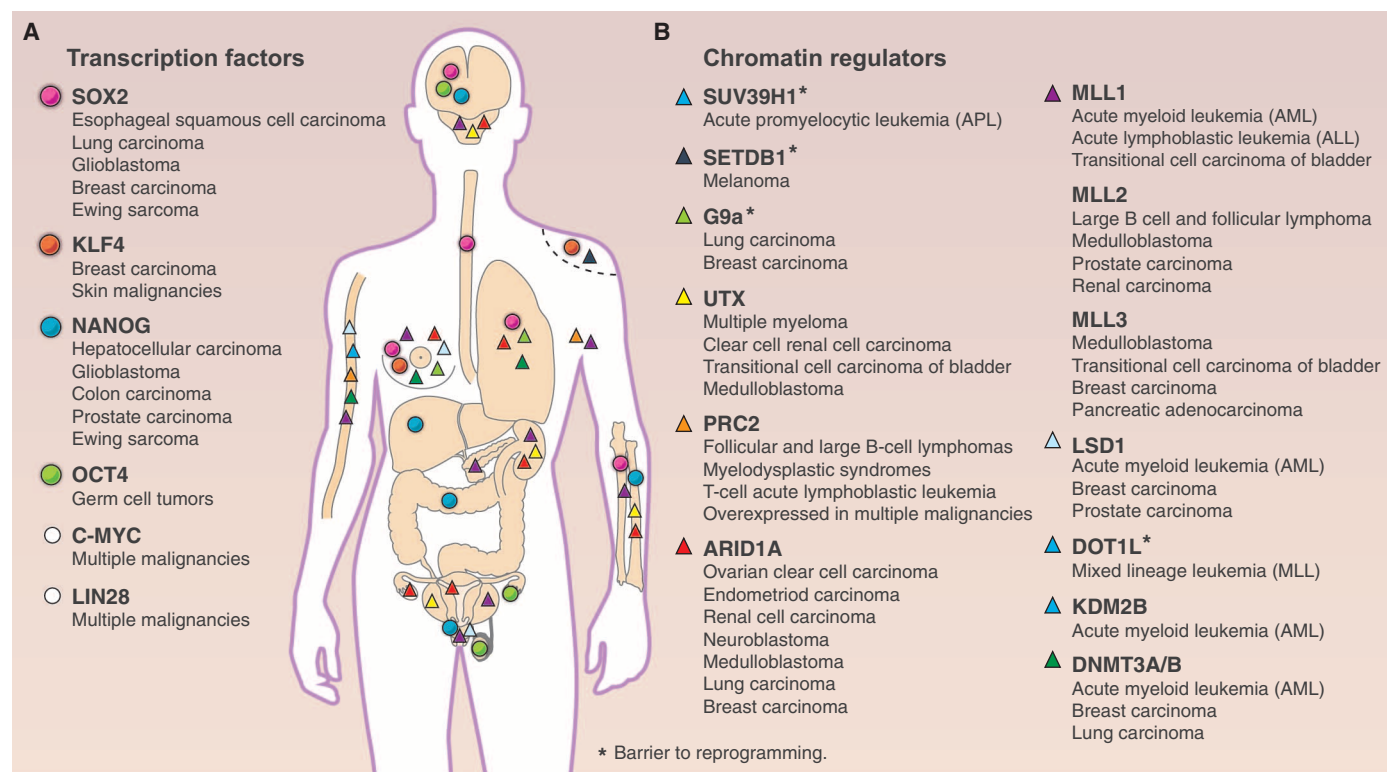


Fig. 2. Genes involved in both iPS nuclear reprogramming and cancer. List of TFs (A) and CRs (B) implicated in iPS reprogramming together with the malignancies in which they have established roles. These include bona fide oncogenes and tumor suppressors directly affected by genetic alterations, as well as other genes with mechanistic roles in cancer.

network while also driving a wide range of malignant programs (24).

Klf4 plays dual roles in cancer, promoting the development of breast and skin malignancies but suppressing gastric, colorectal, and bladder cancer (25). The mechanism by which Klf4 promotes tumor initiation may relate to its functions in nuclear reprogramming. These include functional links to c-Myc and simultaneous repression of p53, both of which enhance cellular proliferation. Klf4 also induces telomerase activity in ES cells and carcinoma cells by promoting β -catenin binding to the *TERT* gene promoter (26). Conversely, suppression of cancer initiation by Klf4 in specific contexts may be mediated through induction of the proapoptotic gene *CDKN1A* (25).

Nanog is expressed in multiple malignancies and appears to have critical functions in cancer stem cell subpopulations. In hepatocellular carcinoma, it is required for CD24-positive stemlike cells, where its expression is maintained by STAT3 signaling (27). In glioblastoma, induction of Gli1 by Nanog increases the clonogenic and tumorigenic potential of the CD133-positive stem cell fraction (28). Nanog is also expressed in stemlike populations in colon and prostate carcinomas, and Ewing sarcoma (Fig. 2A) (20, 29, 30).

Lin28 plays central roles in stem cell biology, accelerating iPS reprogramming and promoting ES cell proliferation (31). This RNA-binding protein is notable for expression across a substantial proportion of human cancers, particularly undifferentiated and advanced malignancies. Lin28 potently inhibits maturation of the Let-7 microRNA family, leading to derepression of multiple oncogenes, including c-Myc, K-Ras, Sall4, and Hmga2.

Recent evidence supports additional mechanisms of action, including a direct effect on Oct-4 translation. These roles may explain the ability of Lin28 to supplement c-Myc in reprogramming and also underlie its oncogenic properties.

In addition to these pluripotency factors, Olig2, Foxg1, and other TFs that mediate direct lineage conversion are implicated in human malignancies, thus generalizing the link between reprogramming and oncogenic transformation (10). It is important to note, however, that the parallels are not complete and that the roles of an individual factor are not necessarily identical in the two processes and may even diverge within different malignant settings. Moreover, the strict analogy to reprogramming fails to appreciate the oncogenic roles of many other TFs, such as transforming fusion proteins identified in hematopoietic malignancies and, more recently, in solid tumors.

DNA Methylation

DNA methylation is a relatively stable epigenetic modification that mediates silencing of repetitive elements and certain gene promoters (32). Methylation patterns must be reset during cellular reprogramming and can present a potent barrier to the process (12, 14). In cancer, hypermethylation of CpG islands is a well-recognized epigenetic event (32). Genetic inactivation of the DNA methyltransferase Dnmt3a has also been documented in leukemia and lymphoma (32, 33). Although the mechanisms remain obscure, DNA methylation may negatively influence transformation by impeding the induction of genes needed for epigenetic reprogramming or, alternatively, may promote oncogenesis by silencing genes that mediate

differentiation or apoptosis. Hence, this epigenetic feature could play markedly different roles in alternate malignant settings. Indeed, seemingly contradictory roles are observed for several CRs in different tumor types, as discussed below.

Chromatin Regulators

Chromatin regulators (CRs) have established roles in cellular reprogramming and oncogenesis (Fig. 2B). Their contributions to malignancy involve multiple modalities. Like specific TFs, many CRs represent bona fide oncogenes or tumor suppressors and are directly affected by gain- and loss-of-function genetic mutations or by translocations (7, 32). Even in the absence of direct genetic alterations, CRs may be co-opted by fusion proteins or other oncogenic factors to modulate gene expression. CRs also regulate other cancer-relevant processes, including epithelial-to-mesenchymal transition (EMT), senescence, genome stability, and metastasis. These diverse modalities and the fact that some CRs have opposite effects in different malignant settings present a challenge for predicting how a given CR mutation, altered chromatin state, or epigenetic therapy will affect a particular tumor.

Repressive Chromatin States

In differentiated cells, inactive portions of the genome are partitioned between different forms of repressive chromatin. Repressive structures with a compact organization refractory to regulatory activity can affect large genomic regions (Fig. 1). Canonical repressive states include classical heterochromatin marked by histone H3 lysine 9 trimethylation (H3K9me3), broad Polycomb-repressed regions enriched for H3K27me3, and highly condensed regions associated with the nuclear lamina and H3K9me2 (4, 6).

H3K9 methylation and associated CRs play important roles in chromosomal stability, EMT, and cellular senescence (32, 34, 35). Large H3K9me3 domains that arise in certain differentiated cells appear to impede the initial binding of reprogramming TFs and may be inefficiently reset during iPS generation (15, 16). Accordingly, suppression of CRs that catalyze H3K9 methylation, including Suv39h1, Setdb1, and G9a, increases reprogramming efficiency (36, 37). H3K27me3 and associated Polycomb repressors play critical roles in tissue-specific gene regulation (38). Ezh2 and Utx, which catalyze the addition and removal of H3K27me3, respectively, are both required for efficient iPS reprogramming (3, 36, 39).

Suv39h1 is required for oncogene-induced senescence, and its loss is associated with decreased viability, genomic instability, and increased tumor risk in mice (35). Recruitment of Suv39h1 to aberrant gene targets by fusion proteins promotes acute myeloid leukemia (40). Setdb1 and G9a have an established role in a number of malignancies (Fig. 2B), and G9a has also been implicated in metastasis and EMT (41, 42). Polycomb regulators

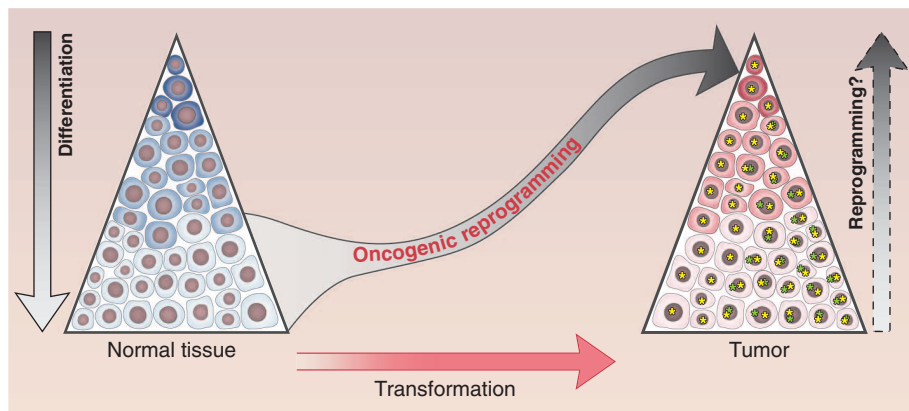


Fig. 3. Cellular hierarchies in normal tissues and malignancies. Normal tissues (left) and a growing list of malignancies (right) have established epigenetic hierarchy, with rare populations of stem cells giving rise to more differentiated cellular progeny through intermediate steps (color shades). Reprogramming experiments have shown that differentiation is reversible (left and right arrows). Cellular transformation (red arrow) is a stepwise process involving accumulation of genetic and epigenetic hits. Once initiated, additional and potentially divergent alterations may occur, establishing a tumor with genetic heterogeneity (illustrated by yellow and green stars) and, within each genetic subclone, an epigenetic hierarchy (color shades). Altered activity of key regulators, including CRs and TFs, can play dual roles in cancer, contributing to transformation and epigenetic state transitions (oncogenic reprogramming). We speculate that the same network of regulators may then act within the established tumor to rewire differentiated cancer cells into stemlike cells, thus establishing a dynamic equilibrium between differentiation and reprogramming.

are subject to genetic mutation or overexpression in a wide range of malignancies (Fig. 2B) (43–49). In particular, Ezh2 and Bmi1 mediate *CDKN2A* epigenetic silencing and are essential in cancer stem cells (7, 32, 38).

Active Chromatin States

Active genes and regulatory elements are associated with characteristic chromatin states. Enhancers and promoters are marked by varying degree of H3K4 methylation and histone acetylation (Fig. 1). The methylation mark is catalyzed by complexes that contain mixed-lineage leukemia (MLL) homologs (related to *Drosophila* trithorax) and accessory subunits such as Wdr5 (7). These CRs play important roles in reprogramming and cancer. Wdr5 directly interacts with the pluripotency TF Oct-4 and is essential for iPS cell generation (12, 50). MLL fusion proteins represent potent leukemic drivers, although they typically lack the catalytic domain and instead appear to function via cofactor recruitment (7). Inactivating mutations of MLL homologs have also been identified in multiple tumors (Fig. 2B). The H3K4 demethylase Lsd1, which functions as a coactivator or corepressor in different contexts (7), also has diverse roles in cancer (Fig. 2B) (7, 51, 52).

The transition from transcriptional initiation to elongation is a critical regulatory step. Dot1l, an H3K79 methyltransferase that promotes this transition, impedes iPS reprogramming (36) and is required for leukemogenesis (53). MLL fusion proteins mediate their aberrant regulatory programs through recruitment of Dot1l. Accordingly, MLL-rearranged leukemias are sensitive to small molecule Dot1l inhibitors (53). The multiple myeloma fusion protein Mmset and Kdm2b respectively catalyze the addition and removal of H3K36 methylation, a characteristic mark of elongating transcripts (7, 54, 55). Kdm2b promotes iPS formation (56), can immortalize primary cells (57), and contributes to the development and maintenance of leukemic stem cells (58).

Many other CRs associated with active chromatin states or chromatin remodeling have been implicated in neoplastic transformation. The CREB binding protein (CBP)/p300 histone acetyltransferase complex functions as a coactivator for specific TFs at proximal and distal regulatory elements, driving epigenetic programs involved in cellular proliferation, apoptosis, differentiation, and DNA stability (59). CBP/p300 also acetylates nonhistone proteins, including p53, Rb, and c-Myc, increasing their transcriptional activity. Chromosomal rearrangements involving CBP/p300 promote hematopoietic malignancies, whereas loss-of-function mutations have been identified in solid tumors, including colorectal and breast carcinomas (7, 59). Dynamic chromatin regulation also requires nucleosome remodeling. The BAF remodeling complex markedly increases reprogramming efficiency by facilitating Oct-4 binding to target loci (60). SWI/SNF homologs, including *ARID1A*, are sub-

ject to recurrent genetic inactivation in a broad range of tumors, although the underlying mechanisms remain unclear (Fig. 2B) (7, 61–63).

These and other studies thus document widespread roles for CRs in human cancer, complementing similarly pervasive functions in development and reprogramming. The fact that CRs with similar catalytic activities and developmental phenotypes can lead to apparently contradictory outcomes in different malignant settings may relate to their diverse roles in gene regulation, differentiation, senescence, telomere regulation, and other processes. The same CR might function in alternate contexts to facilitate epigenetic reprogramming, to arrest the epigenetic state of a progenitor cell by differentiation block or to counteract apoptosis or senescence. Substantial variability in the epigenetic makeup of premalignant target cells or the maintenance requirements of a particular tumor may also help explain varied and sometimes incongruous roles for TFs and CR mutations across the spectrum of human cancers.

From Reprogramming to Cancer Stem Cells and Beyond

Here, we have explored conceptual parallels between reprogramming and cancer, and highlighted shared TFs and CRs unveiled by mechanistic studies and cancer genome sequencing. The analogy to reprogramming suggests that some CR alterations in cancer may represent early events that render a cell of origin susceptible to epigenetic rewiring required for transformation and refractory to differentiation, proliferation arrest, or apoptosis. Subsequent hits, occurring in variable orders and combinations, may then confer the definitive transformed state and the dynamic cellular hierarchy within a tumor (Fig. 3). In certain cancers, stemlike cells critical for tumor initiation and growth occupy the apex of this hierarchy (64). Just as the reprogramming field is deciphering the combinatorial TF code for cellular identity, cancer research is increasingly focused on the determinants of heterogeneous epigenetic states in tumors. By activating specific TF drivers and modulating collaborating CRs, cancer cells may dynamically regulate their epigenetic circuits to rewire differentiated cancer cells into stemlike cells, thus refueling cancer growth. Although speculative, such dynamic bidirectional transitions could provide a unifying view of cellular organization within tumors, compatible with both the cancer stem cell and the stochastic models. Regardless of which model best explains a given malignancy, principles and mechanisms shared between cellular reprogramming and oncogenic transformation provide fundamental insights into tumor biology with the potential to guide biological understanding, diagnosis, and therapeutic strategies.

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Dust and Biological Aerosols from the Sahara and Asia Influence Precipitation in the Western U.S.

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Winter storms in California's Sierra Nevada increase seasonal snowpack and provide critical water resources and hydropower for the state. Thus, the mechanisms influencing precipitation in this region have been the subject of research for decades. Previous studies suggest Asian dust enhances cloud ice and precipitation, whereas few studies consider biological aerosols as an important global source of ice nuclei (IN). Here, we show that dust and biological aerosols transported from as far as the Sahara were present in glaciated high-altitude clouds coincident with elevated IN concentrations and ice-induced precipitation. This study presents the first direct cloud and precipitation measurements showing that Saharan and Asian dust and biological aerosols probably serve as IN and play an important role in orographic precipitation processes over the western United States.

Aerosols can modify cloud microphysical properties, including droplet size and water phase, and thus can alter precipitation efficiency (1). In particular, dust aerosols, which originate from various deserts around the world (2), have been shown to serve as effective ice nuclei (IN) and potentially enhance precipitation in mixed-phase clouds (3). IN are atmospheric particles that catalyze the freezing of supercooled cloud droplets, producing ice crystals that would not form otherwise at warmer, mixed-phase cloud temperatures (4). Some types of biological aerosols such as bacteria have also been shown to serve as effective IN at relatively warm temperatures (5); however, recent modeling studies have concluded that they are of minor importance to global IN concentrations and precipitation processes (6). Biological aerosols (such as bacteria), can be co-lofted and transported with dust (7). When lofted to high altitudes (≥ 5000 m), dust and biological aerosols can travel long distances. For example, Uno *et al.*

showed that dust from the Taklimakan desert in China circled the globe within 13 days (8). Intercontinental transport of dust from Asia is well documented (9–12), whereas few have reported trans-Pacific transport from North Africa (13, 14). Modeling studies have shown that both Asian and African dust influence ice formation in mixed-phase clouds (15, 16). Small amounts of dust can be lofted into the free troposphere from the Taklimakan as early as February, with maximum concentrations detected between April and May. Much of the Gobi desert is frozen in the winter months, but it can represent a substantial Asian dust source in response to strong winter storms from Siberia (3). Dust is lofted from the Sahara year round, so this source region has the potential to affect ice formation in clouds throughout the year (3).

IN concentrations are generally low (~ 1 in every 10^5 aerosol particles in the free troposphere at -20°C) (17), highly variable, and strongly dependent on the chemical composition of the aerosols present within a particular cloud (18). At mixed-phase cloud temperatures (above roughly -36°C), IN are necessary for ice formation. Ice crystals grow diffusively at the expense of liquid droplets (19) and can gain mass through accretion of supercooled liquid droplets or aggregation with other crystals to form graupel or snow (20, 21). Freezing of cloud droplets induced by IN produces a mixed-phase cloud that initiates precipitation more rapidly than does a supercooled, liquid-only cloud because of the faster growth rate of ice particles versus droplets (22). The presence, absence, and abundance of IN can thus affect the intensity and spatial distribution of precipitation events. The efficiency of ice and precipitation processes has serious ramifications for mountainous regions such as the California Sierra Nevada, where snowpack

supplies copious amounts of water to reservoirs (23). Hence, cloud-seeding experiments have been conducted in the Sierra Nevada since 1948 as a possible means of increasing precipitation (24, 25). It has been suggested that over 50% of precipitation globally is initiated in the ice phase [such as in (26)]. Therefore, identifying the sources of IN within clouds and the mechanisms by which they influence precipitation processes is critical for future water and energy.

Analysis of precipitation samples in combination with storm meteorology can provide insight into IN effects on orographic precipitation. For example, Ault *et al.* observed insoluble residues in precipitation samples collected in the Sierra Nevada, which were hypothesized to be Asian dust (1). There are two key unresolved questions from the Ault *et al.* study: (i) Do the insoluble precipitation residues reflect the composition of the IN that initially nucleated cloud ice? (ii) What role does dust play in affecting cloud microphysics and precipitation? Further evidence from Pratt *et al.* provided aircraft measurements of dust and biological residues co-located with ice in clouds over Wyoming, but this was a limited data set, with no emphasis linking observations with precipitation (27). Here, we present results building on those from Ault *et al.* and Pratt *et al.* using a combination of both ground-based precipitation and aircraft in situ cloud measurements in California throughout the 2011 winter. Our results indicate a relationship between dust and biological aerosols detected in in situ cloud residues in distinct mid-level ice layers, residues in precipitation collected at the ground, and long-range transport of dust and biological aerosols from Asia, the Middle East, and the Sahara. Combined, these results strongly suggest dust and biological aerosols affected ice formation in mid-level clouds where precipitation processes were initiated.

The CalWater field campaign was designed to directly address aerosol impacts on clouds and precipitation in the Sierra Nevada during three consecutive winter seasons (2009 to 2011). Measurements from a remote ground site at California's Sugar Pine Dam [SPD, $39^\circ 07' 42.80''\text{N}$, $120^\circ 48' 04.90''\text{W}$; 1064 m mean sea level (MSL)] included aerosol and meteorological instrumentation from 2009 to 2011. The same technique used by Pratt *et al.* (27) and Ault *et al.* (1)—aerosol time-of-flight mass spectrometry (AOFMS) (28)—was used to determine the chemical composition of resuspended insoluble precipitation residues collected at SPD from 30 January to 8 March 2011. S-band profiling radar (S-PROF) provided bulk microphysical information using vertical profiles of hydrometeor fall velocity and radar reflectivity (29). Precipitation processes included warm rain, which started as liquid; cold rain, which started as ice and melted during descent; and snow/graupel/hail when surface temperatures were low (30). Precipitation that starts in the ice phase—snow/graupel/hail and cold rain—

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is termed “ice-induced precipitation.” S-PROF time-height cross sections during the times for all 11 of the precipitation sampling periods are shown in fig. S1.

During all of the 2011 storms, dust and biological residues were frequently present in the precipitation, comprising up to 99% of the total insoluble residues per sample. The percentage of dust plus biological residues (%Dust+Bio), median cloud top temperature ($^{\circ}\text{C}$), relative amount of cloud ice (%Ice), and percentages of different precipitation processes during each sample collection date in 2011 are shown in Fig. 1. Dust and biological residue percentages were combined for the precipitation analysis because chemical markers for each type were often observed within the same individual residue owing to the precipitation collection and aerosolization processes (fig. S2 and supplementary text). Ice-induced precipitation comprised 74% of the total precipitation that fell at SPD, whereas warm rain comprised only 10% (the remaining 16% was

unclassified). The highest %Dust+Bio in precipitation samples occurred during storms that contained a higher percentage of ice-induced precipitation (such as 30 January, 16 to 19 February, and 24 to 26 February). During these storms, surface temperatures were sufficiently low (table S1), enabling snow/graupe/hail to reach the surface. During the storms from 14 to 16 February, 1 to 3 March, and 5 to 7 March, surface temperatures were higher, resulting in more rain than ice at the surface. Further, more warm rain coincided with lower %Dust+Bio during these time periods. For the five samples in which >50% of precipitation fell as snow/graupe/hail, the average %Dust+Bio was 90%, whereas for the four cases in which >30% of the precipitation occurred as warm rain, the average %Dust+Bio was 69%. One possible explanation could be that a limited amount of dust and biological aerosols were available to serve as IN, leading to less precipitation initiated in the ice phase. Another potential explanation is that the thermodynamic

and dynamic structure of the storm produced shallower clouds (fig. S1) with warm rain, which were perhaps unable to reach vertically into the dust plume. Typically, the cloud top temperatures were lower when the %Dust+Bio in precipitation and %Ice in cloud were higher, suggesting that dust and biological aerosols are more efficiently activated and scavenged via ice nucleation at colder temperatures. Both the 1 to 2 March and 2 to 3 March time periods had similar cloud top temperatures (-22°C); however, the %Dust+Bio and %Ice were higher on 1 to 2 March and lower on 2 to 3 March. Thus, this result suggests that the formation of in-cloud ice is not entirely dependent on the cloud temperature alone and that the presence and abundance of IN feeding the clouds is important. Overall, these results suggest that dust and biological aerosols probably served as IN and influenced the precipitation phase in the clouds within colder sectors of the storms, expanding on the precipitation measurements made by Ault *et al.* in 2009. Precipitation residues can provide useful information on the nature of aerosols and cloud seeds; however, because of the caveats involved with analyzing precipitation and correlating ground-based measurements with mid-level cloud processes, in situ aircraft measurements of cloud residues made during CalWater 2011 were critical for gaining further insight into the role of the dust and biological particles in cloud and precipitation processes.

A compact aircraft version of the ATOFMS (31) measured in situ cloud droplet and ice crystal residues onboard the G-1 during CalWater. Cloud residue data during each precipitation sampling time period are included in Fig. 1. Discussion regarding the collection of dust residues and the relative amount of dust and biological residues in the precipitation compared with the cloud residues is provided in the supplementary materials. High percentages of dust and dust mixed with biological cloud residues were observed during the flights on 16 and 25 February, respectively. On 16 February, the cloud residues were rich in aluminosilicate dust (50%), with smaller contributions from dust mixed with biological material (“dust/biological,” 16%) and purely biological residues (3%). However, on 25 February, residues were predominantly composed of dust mixed with salt and biological material (“dust/salt/biological,” 66%), whereas dust and biological residues represented 9 and 10%, respectively. The precipitation residues were also characterized as more “dustlike” on 16 February and were composed of biological material on 25 February (supplementary text). Although the precipitation residue compositions were not exactly the same as the cloud residues, the similarities between the two suggest that the majority of precipitation residues probably originated from the cloud particles as opposed to being scavenged by precipitation in the air below cloud base. The meteorological conditions were similar during these 2 days and corresponded

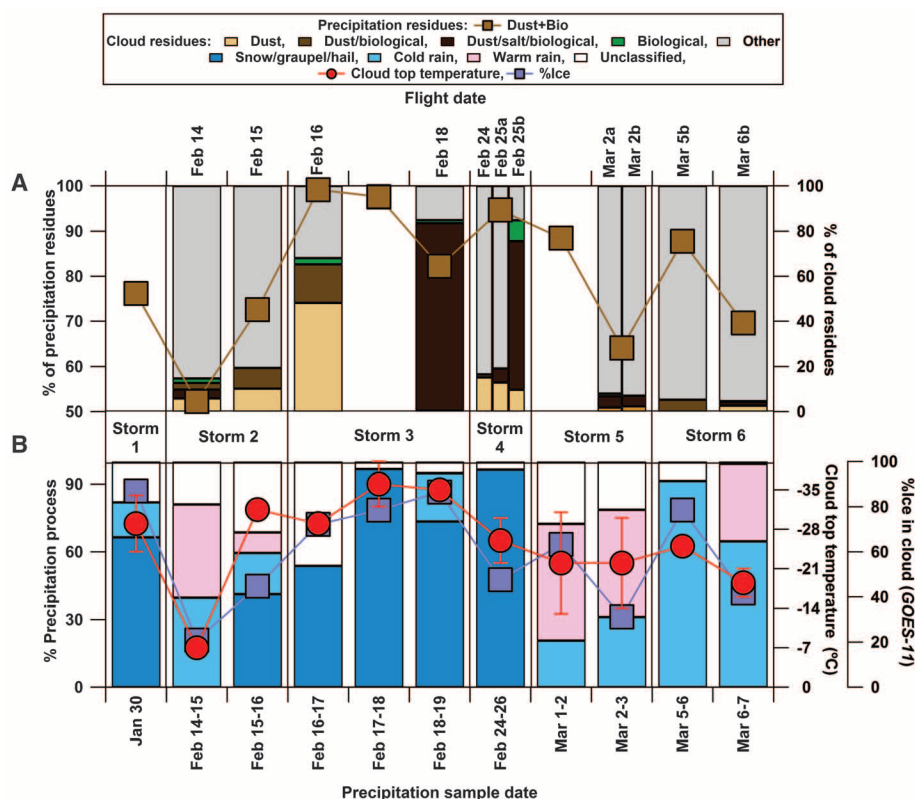


Fig. 1. Precipitation and cloud characteristics during each sample collection date during the 2011 CalWater study at SPD. (A) Markers show the percentages of dust and biological precipitation residues combined (Dust+Bio) sampled at the surface at SPD, and bars show the percentages of in-cloud residues sampled on the G-1 aircraft. Sample dates with more than one column of bars for the aircraft data signify more than one flight took place; for instance, three flights were taken 24 to 26 February and two flights were taken 2 to 3 March. (B) The percentages of cold rain, snow/graupe/hail, and warm rain per sample (taken from S-PROF radar profiles). Red markers represent the median cloud top temperature (in degrees Celsius) per sample collection date. Error bars are shown for temperature and represent the minimum and maximum of the range. Violet markers represent the average relative amount of the cloud consisting of ice (%Ice) within the 10-km radius over SPD derived from satellite measurements. Storms 1 to 6 are highlighted in order to identify the evolution of cloud and precipitation residues, cloud top temperature, %Ice, and precipitation processes over the course of the storms.

to the passing of a cold front (supplementary text). The passing of these cold fronts probably enabled the transport of dust and biological aerosols into the region, as suggested by previous studies (1). However, if IN were hypothetically absent in these cold air masses, supercooled cloud droplets would remain as liquid instead of forming ice crystals, as shown by Rosenfeld *et al.* (32) and described here. On days without these meteorological conditions, we observed only minor fractions of dust and biological particles in cloud residues (fig. S4), showing that dust and biological particles are not always present. Additionally, estimated dust concentrations detected between 3000 and 6000 m are provided and discussed in the supplementary materials to show that the variability of dust concentrations can range over an order of magnitude during flights. This strongly supports the concept proposed here that ice formation depends not only on the meteorological conditions but also on the availability of effective IN, such as certain mineral dusts and biological particles.

In order to investigate the impact of dust and biological particles on cloud properties during the storms on 16 to 19 February and 24 to 26 February, we examined data from the flights corresponding to these storms in more detail. Shown in Fig. 2 are the number of individual cloud residues measured by A-ATOFMS for the 16 February ascent (Fig. 2A) and 16 February descent (Fig. 2B) flight segments (flight tracks are available in the supplementary materials). The 25 February ascent and descent are shown in fig. S8. Flights on 16 and 25 February are separated into the ascent and descent because of changing dynamics (supplementary text). The early mornings on 16 and 25 February included prefrontal periods with an atmospheric river (narrow band of water vapor transported from subtropical oceanic regions) and terrain-parallel blocked flows (supplementary text), which introduced moist marine air masses into deep clouds over the Sierra Nevada (fig. S1). In the afternoon during the flights on 16 and 25 February, cold postfrontal conditions with lower-level convective clouds and mid-level orographic clouds were present. Liquid water content (LWC), the mass concentration of liquid in a cloud, was measured with a cloud droplet probe (CDP). IN concentrations (IN per liter of air) were measured with a continuous flow diffusion chamber (CFDC) (17). Cloud ice fraction was calculated by using liquid and total water content measured with the WCM-2000 (SEA, Mansfield Center, Connecticut). Details of the calculation are available in the supplementary materials.

During the ascent to high altitudes on 16 February, the lower-level convective clouds (≤ 3500 m) were pristine marine clouds that contained predominately sea salt residues and few dust particles (Fig. 2A). Correspondingly, the IN concentrations were low (0 to a maximum of 0.15 ± 0.2 liter $^{-1}$ in a temperature range from -18.4 to -18.9°C). These measurements were consistent with little to no

ice in-cloud as evidenced by a low cloud ice fraction (0 to 0.4) and high LWC (up to ~ 1 g/m 3). The nearly ice-free, supercooled cloud reached temperatures down to -21°C . The presence of liquid water and lack of large amounts of ice was consistent with very few IN active in this region of the cloud. The IN concentrations in the mid-level orographic clouds (4000 to 6000 m) could be determined with greater certainty at 0.16 ± 0.06 liter $^{-1}$ measured at -18.8°C , whereas the cloud top temperature reached -35°C and contained ice along with supercooled water of <0.1 g/m 3 . In addition, a spike in dust and dust/biological residues was observed between 4400

and 5500 m in these mid-level clouds. Transmission electron microscopy (TEM) analysis showed that 88% of IN-activated aerosol in the CFDC was of dust and biological origin (53% dust, 26% dust/salt/biological, and 9% biological)—thus confirming the dominance of dust and biological cloud residues observed by the A-ATOFMS, as discussed above. However, above 5500 m we observed less dust and biological residues coincident with supercooled liquid water at temperatures as low as -35°C , thus showing a distinct layer of dust and biological aerosols played a role in forming the ice-enriched layer at lower altitudes and warmer temperatures. During the

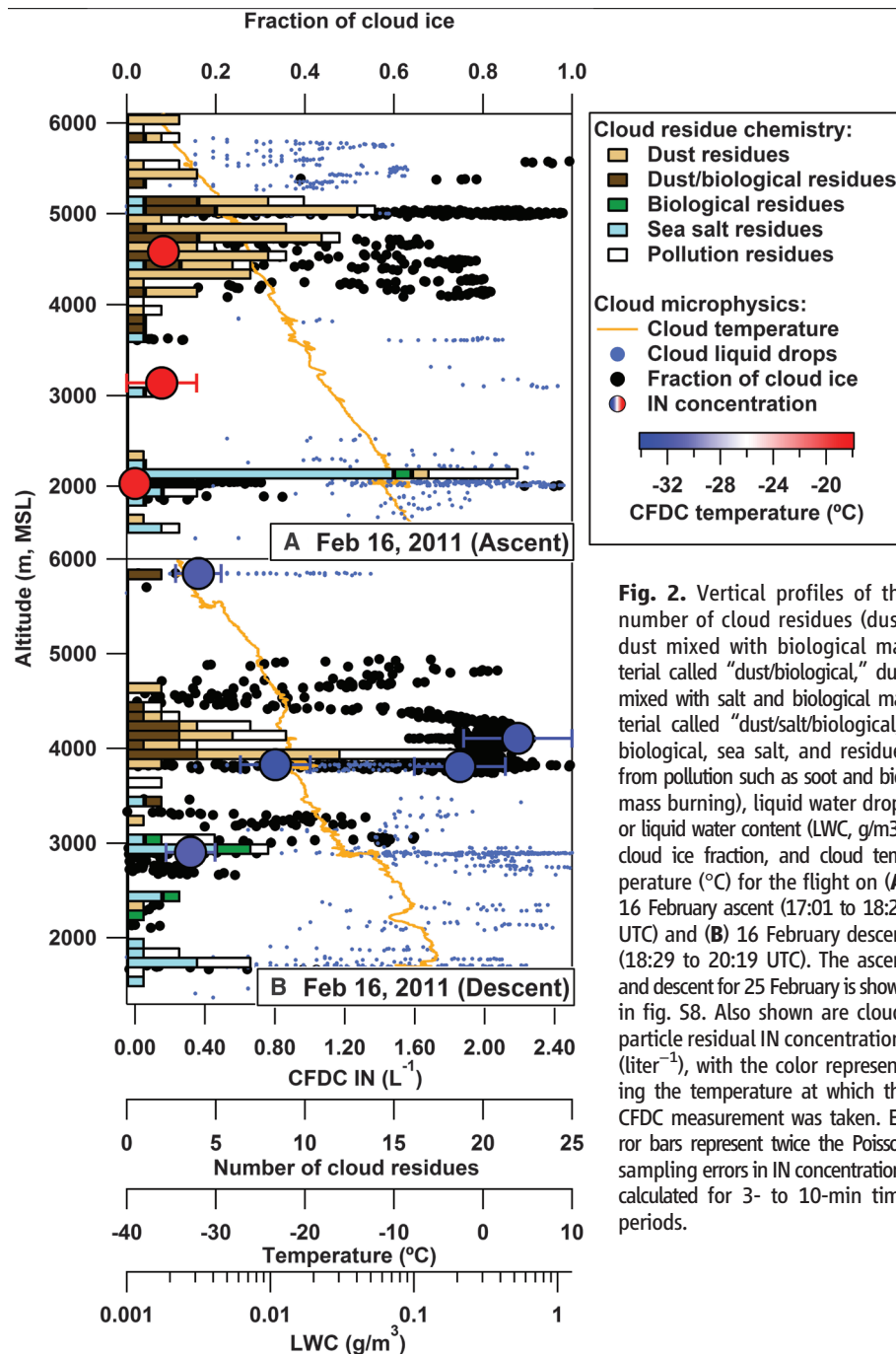


Fig. 2. Vertical profiles of the number of cloud residues (dust, dust mixed with biological material called “dust/biological,” dust mixed with salt and biological material called “dust/salt/biological,” biological, sea salt, and residues from pollution such as soot and biomass burning), liquid water drops or liquid water content (LWC, g/m 3), cloud ice fraction, and cloud temperature ($^\circ\text{C}$) for the flight on (A) 16 February ascent (17:01 to 18:29 UTC) and (B) 16 February descent (18:29 to 20:19 UTC). The ascent and descent for 25 February is shown in fig. S8. Also shown are cloud-particle residual IN concentrations (liter $^{-1}$), with the color representing the temperature at which the CFDC measurement was taken. Error bars represent twice the Poisson sampling errors in IN concentrations calculated for 3- to 10-min time periods.

descent back down to lower altitudes (Fig. 2B), dust and dust/biological cloud residues were detected at several altitudes but were most abundant between 3500 and 4500 m, where the highest ice fractions were observed (up to 1). IN concentrations measured at a lower CFDC processing temperature of $-32 \pm 0.5^\circ\text{C}$ trended with the abundance of the dust and biological residues, reaching $2.2 \pm 0.3 \text{ liter}^{-1}$ between 4000 and 4500 m. The large increase in IN concentrations measured in dust layers at -32°C on the descent

as compared with those at -18.4° to -18.8°C on the ascent suggests that the dust was IN-active within the intermediate temperature range. The co-location of these specific residues with ice crystals and higher IN concentrations suggest dust and biological aerosols served as IN in these mid-level clouds.

On 25 February, the meteorological and cloud conditions were similar to those of 16 February. However, dust/salt/biological aerosols as opposed to pure dust on 16 February were co-located with

ice, particularly around 2700 and 3900 m. The residues were separated into two distinct layers, as shown in fig. S8A: a lower layer from 1400 to 3100 m and an upper layer from 3800 to 4000 m. IN concentrations reached $0.39 \pm 0.2 \text{ liter}^{-1}$ measured at -25.0°C in the upper layer, whereas in the lower layer, maximum IN concentrations of $0.51 \pm 0.24 \text{ liter}^{-1}$ were measured at -25.3°C ; the CFDC temperatures were appropriate for the upper layer (-26° to -20°C) but colder than cloud temperatures in the lower layer (-17° to -10°C). The mixed nature of these particles makes it difficult to extract which component (dust or biological) enabled the ice formation. The observations on 16 and 25 February bracket the activation temperatures of the dust and biological particles between -18° and -32°C , with most of the activity occurring below -25°C . This is in agreement with previous laboratory studies, showing that many mineral dusts are more IN-active below $\sim -20^\circ\text{C}$, whereas biological particles such as spores become active at $\sim -28^\circ\text{C}$ (32), as well as with satellite observations of Asian desert dust glaciating cloud tops at -22°C on average (3).

On 25 February, the mid-level cloud contained supercooled cloud water of up to 0.3 g/m^3 , with some ice crystals that rimed into graupel at lower levels (supplementary text), suggesting that the ice was formed in the mid-level cloud and fell into the lower-level cloud while riming and depleting the cloud water. These results, along with the results on 16 February, are indicative of the seeder-feeder mechanism (33), which has been documented previously in the Sierra Nevada (34) as well as in other mountain regions including those in Colorado, Oregon, Washington, Arizona, and central Europe (35–38). These data suggest that particles with biological components, in addition to dust, play a role in ice formation in the orographic clouds in the Sierra Nevada and contribute to the efficiency of the seeder-feeder mechanism. Previous studies have shown that a graupel particle grows much faster than does a supercooled raindrop of the same mass in the same convective cloud (22). Therefore, seeder-feeder is an efficient precipitation mechanism, especially when the accreted drops are large (39). Satellite observations have suggested the possibility of dust glaciating high-altitude clouds (40); however, there have not been any in situ measurements of dust in clouds over the Sierra Nevada before this study. When urban pollution aerosols are incorporated into clouds, precipitation processes are often less efficient, and suppression of both warm and mixed-phase precipitation has been shown via satellite (41, 42). Our results show that the presence of dust and biological particles feeding deeper and colder orographic cloud levels appears to have the opposite effect—accelerating the efficiency of the precipitation-forming processes, especially in situations in which cloud layers are decoupled from the boundary layer, likely because of the presence of terrain-parallel blocked flow (supplementary text). To address this, future studies

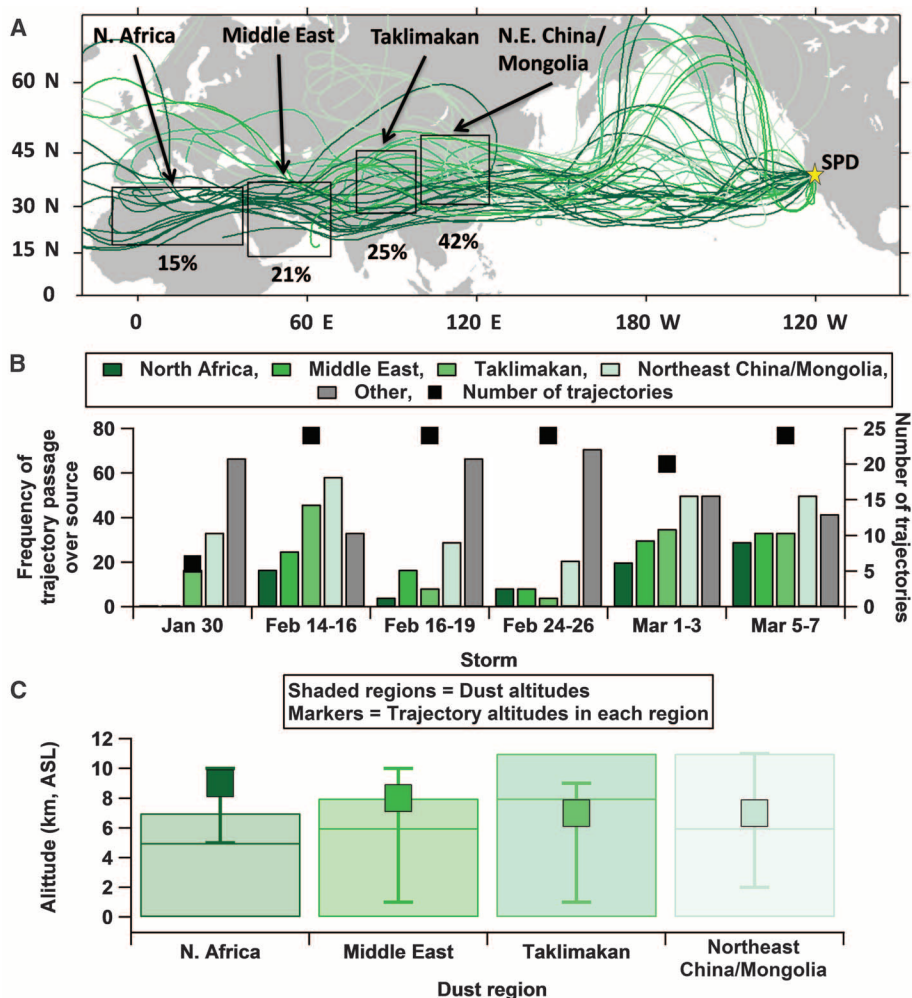


Fig. 3. Dust source analysis including (A) 10-day back trajectories ending at cloud top heights over SPD during storms in 2011, with the boxes highlighting the four dust source regions. Trajectories are colored according to which dust region they traveled over, including the Sahara (separated into North Africa and the Middle East) and East Asia (separated into the Taklimakan desert and Northeast China/Mongolia). Storms were investigated in order to achieve statistical significance (more trajectories per time period). The percentages under each box represent the frequency that the trajectories passed through each arid region. (B) The frequency with which 10-day back trajectories passed over the dust regions defined in (A) in addition to other regions (nondust origin). The four shades of green represent the four different dust regions. The total number of trajectories analyzed per storm is also provided. (C) The average altitudes of trajectories that passed through each region and dust layer heights determined by using NAAPS analysis time-height sections at Sedebocker (North Africa), Solar Village (Middle East), Yinchuan (site nearest to the Taklimakan), and Beijing (Northeast China/Mongolia) for the time periods studied. A map of the sites and examples of the time-height sections are shown in fig. S11. Data originate from time periods that include back trajectory end points shown in fig. S13. The middle line of the shaded regions represents the average maximum height of the dust layers in each region from NAAPS analysis. The error bars show the minimum and maximum altitudes for the trajectory end points and dust layers.

should be designed to observe both influences, boundary-layer and upper-level aerosols, as well as associated meteorological conditions.

The co-location of dust and biological aerosols with IN and ice in clouds, in addition to the abundance of dust and biological residues observed on days with more ice-induced precipitation during CalWater, leads to a question of the origin of the dust and biological aerosols. Ault *et al.* suggested Asian dust influenced snowfall during only one storm in 2009 (9); however, we present here six storms with influences from Asia and the Sahara. Local sources of dust and biological aerosols were probably minor contributors to the precipitation residues (supplementary text). Ten-day air mass backward trajectories are shown in Fig. 3A, calculated with HYSPLIT (43) ending at SPD during the storms shown in Fig. 1. Meyers *et al.* suggest that ice nucleation occurs at the tops of orographic clouds (34), and thus trajectories were calculated to end at cloud top heights between 2000 and 10,000 m MSL over SPD by using data from the 11th Geostationary Operational Environmental Satellite (GOES-11) averaged every 3 hours during storms. The boxes highlight major dust regions (2). Based on previous studies [such as (44)], some biological aerosols were probably co-lofted from arid regions and transported with the dust, although other sources of biological material such as the ocean and local sources could also contribute. The frequency of trajectories that traveled over each dust region during each storm is shown in Fig. 3B. Trajectories that originated from over North Africa frequently traveled over the other dust regions on the way to the United States, suggesting that these air masses contained a mixture of dust from the various source regions. Further, wet and dry deposition of the dust and biological aerosols could contribute to loss during transport from the more distant sources (Africa). The dust source frequencies show that the storms were predominantly affected by Asia; however, the end of the study was additionally influenced by North Africa and the Middle East (15 and 21% of the time, respectively). Not only did the air masses travel over the various dust regions, the air intersected dust layers (Fig. 3C). Using time-height cross sections of dust concentrations from the Navy Aerosol Analysis and Prediction System (NAAPS, www.nrlmry.navy.mil/aerosol/), we estimated the maximum height of dust layers at sites within or near each dust region on days during which the trajectories traveled over the boxes. The height of trajectories that passed through each region typically fell within the height of the dust layers (Fig. 3C). Although transport within the dust layers was infrequent over North Africa, Saharan dust was probably picked up over the Middle East, where trajectories, on average, skimmed the top of the layer. Overall, trajectories traveled through dust layers 6, 23, 73, and 45% of the time in North Africa, the Middle East, the Taklimakan, and Northeast China/Mongolia, respectively (fig. S13).

Further evidence that dust was present along the complete trajectory was obtained by using Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observation (CALIPSO) satellite data of aerosol subtypes. In combination with NAAPS, CALIPSO provides a method to track dust as it is transported from the source across the Pacific Ocean to SPD (fig. S14). NAAPS-modeled aerosol optical depth (AOD) of dust is shown in Fig. 4, in addition to locations where CALIPSO imagery transected the trajectories for 16 and 25 February. These two days represent the storms in which the highest %Dust+Bio was present in the precipitation (93 and 95% on average during 16 to 19 February and 24 to 26 February). The trajectory ending on 16 February started 10 days earlier over the Atlantic Ocean and proceeded to travel north of the Saharan dust layer. Before reaching China, the air mass contained little dust according to CALIPSO imagery (Fig. 4A, the “No Dust” circle). Once the air mass reached Northeast China/Mongolia, it picked up fresh dust and traveled across the Pacific Ocean to SPD. In contrast, the trajectory ending on 25 February started 10 days back over Oman, where an abundance of fresh dust was present. The air mass continued to travel over southern China, where it probably picked up more dust from the Asian sources. The CALIPSO imagery following the trajectory over the Pacific Ocean shows both fresh and polluted dust. Combined analysis of air mass back trajectories, NAAPS, and CALIPSO imagery provides evidence of lofted dust being transported across the Pacific Ocean

and shows the probable sources of IN that influence precipitation processes in the Sierra Nevada. Conventionally, dust from the Sahara has been shown to travel west over the Atlantic Ocean from Africa to South America; however, here we show African dust traveling east across the Pacific and into the United States at higher altitudes.

We present a direct link between long-range transported dust and biological aerosols affecting cloud ice formation and precipitation processes in the Sierra Nevada. As summarized in Fig. 5, long-range transport of the dust and biological aerosols is shown to be an important mechanism for introducing IN into mid-level clouds and enhancing precipitation formation via seeder-feeder in the Sierra Nevada. Developing this whole conceptual picture requires combining evidence from multiple measurements, including (i) ground-based precipitation chemistry, (ii) satellite data showing dust activity and transport, (iii) aircraft in situ cloud microphysics and residual aerosol composition, (iv) detailed meteorology at the ground level as well as within the clouds, and (v) out-of-cloud aerosol measurements ($>0.6 \mu\text{m}$). This study is the first to show dust originating from further west than Asia plays a role in clouds and precipitation processes over the western United States. Dust and biological aerosols measured as insoluble residues in precipitation samples corresponded to periods with more ice-induced precipitation. In situ aircraft measurements of the same storm systems confirmed that the dust and biological

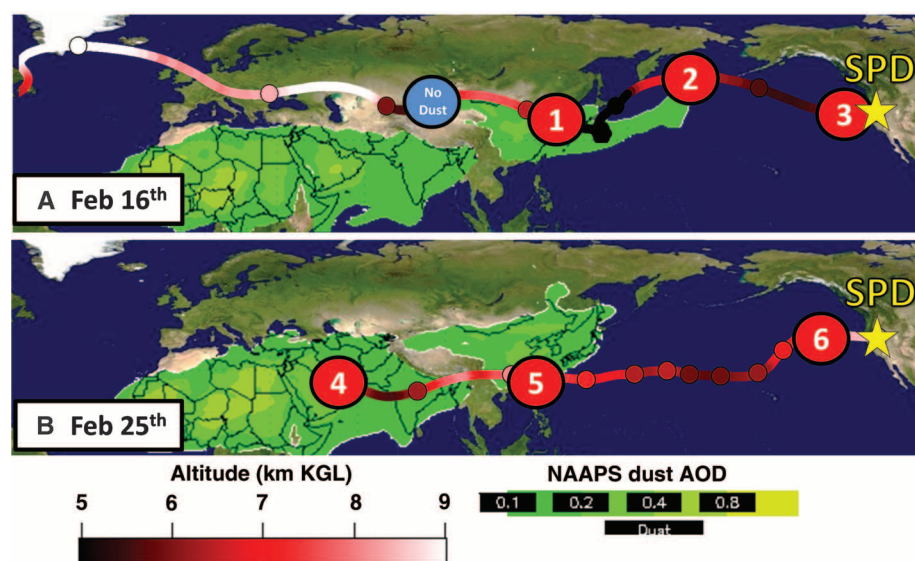


Fig. 4. Dust tracking for days during the (A) 14 to 16 February and (B) 24 to 26 February storms. The 10-day air mass back trajectories ended at cloud-top heights over SPD (shown by the star) on 16 February (7390 m MSL) and 25 February (9340 m MSL). Within each trajectory, the color scale represents the altitude of each hourly end point, whereas the circles show each end point at 00:00; each marker represents 1 day back. The last 2 days from the trajectory ending on 16 February are not shown. The circled numbers mark the locations where dust was present along these trajectories determined by using CALIPSO imagery (fig. S14). The green overlays represent dust AOD from 0.1 to 0.8 modeled by NAAPS. The dust AOD overlays are composites of the 10 days during each trajectory: (A) 6 to 16 February and (B) 15 to 25 February.

precipitation residues were co-located in a distinct cloud layer, with higher IN concentrations and larger fractions of ice relative to supercooled liquid. Many factors contribute to the type and quantity of precipitation, including dynamics, meteorological forcings, and transport conditions; however, our results suggest that dust and biological particles play a key role in ice formation and potentially precipitation initiation by causing glaciation of mid-level clouds. Further, our results also suggest that biological particles may play a larger role than modeling studies suggest, which indicates further studies are needed on the surface emission rates, oceanic fluxes, and IN efficiency of biological aerosols, of biological aerosols, especially when mixed with other species. In situ cloud measurements such as these

provide critical insights into the key chemical species involved in the formation of ice in clouds; however, controlled laboratory studies are needed to better understand the mechanisms involved as well as sort out critical chemical details, such as whether metals, minerals, biological material, or some combination control the initial ice formation process.

Before this study, most studies had focused on modeling the impacts of regional aerosol pollution sources on precipitation. Our results demonstrate the potentially critical impact of intercontinental transport of natural aerosols on precipitation-formation processes. Further, these findings motivate the challenging study of quantifying aerosol effects on not only the initial cloud phase and formation of precipitation but also the

intensity and location of precipitation. Such studies must also quantify the role of non-aerosol-related meteorological forcings of orographic precipitation. Because of the ubiquity of dust and biological particles such as bacteria in the atmosphere, these findings have global importance. Furthermore, the implications for future water and energy resources from hydropower become even more substantial when considering the possible increase in Aeolian dust as a result of a warming climate and land-use changes.

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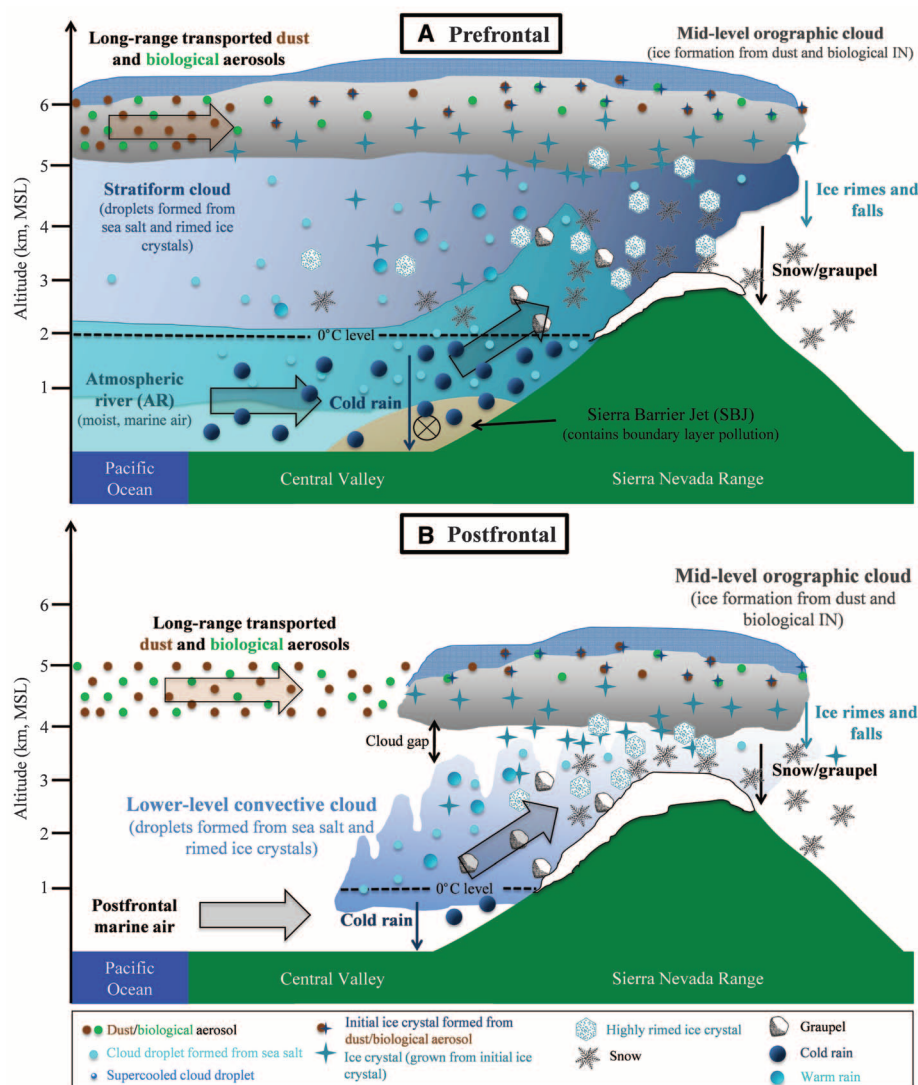


Fig. 5. Conceptual model summarizing results from (A) prefrontal and (B) postfrontal conditions presented here. Prefrontal conditions corresponded to a deep continuous cloud layer, atmospheric river (AR), and terrain-parallel blocked flow [Sierra Barrier Jet (SBJ)], which shifted to two cloud layers, and the disappearance of the AR and blocked flow during the postfrontal conditions. Dust and biological aerosols are long-range transported and form ice in mid-level orographic clouds. The ice becomes rimed by supercooled drops and falls into lower-level convective clouds formed in pristine, marine air masses. Thus, precipitation is formed via the seeder-feeder mechanism.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S15
Tables S1 to S3
References

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Multiple Instances of Ancient Balancing Selection Shared Between Humans and Chimpanzees

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Instances in which natural selection maintains genetic variation in a population over millions of years are thought to be extremely rare. We conducted a genome-wide scan for long-lived balancing selection by looking for combinations of SNPs shared between humans and chimpanzees. In addition to the major histocompatibility complex, we identified 125 regions in which the same haplotypes are segregating in the two species, all but two of which are noncoding. In six cases, there is evidence for an ancestral polymorphism that persisted to the present in humans and chimpanzees. Regions with shared haplotypes are significantly enriched for membrane glycoproteins, and a similar trend is seen among shared coding polymorphisms. These findings indicate that ancient balancing selection has shaped human variation and point to genes involved in host-pathogen interactions as common targets.

Balancing selection is a mode of adaptation that leads to the persistence of variation in a population or species in the face of stochastic loss by genetic drift. In humans, examples include the sickle cell hemoglobin polymorphism, maintained by heterozygote advantage in environments in which *Plasmodium falciparum*

is endemic, as well as other cases that likely arose recently in evolution in response to malaria (1). Beyond humans, examples of balancing selection are known in a wide range of organisms and often seem to arise from predator-prey or host-pathogen interactions (2–8). Most are not thought to be due to heterozygote advantage but to negative frequency-dependent selection, as occurs at self-incompatibility loci in plants (5, 9), or to temporally or spatially varying selection, as seen at R genes in *Arabidopsis*, for example (4). The genetic basis is known only in a small subset of cases, however, and the age-old question (10–12) of how much genetic variation is maintained by balancing selection remains largely open.

When balancing selection pressures result in the stable maintenance of genetic variation in the population for long periods of time, neutral diversity accumulates at nearby sites; in other words, ancient balancing selection leads to deep coalescence times to a common ancestor at the selected site (or sites) and closely linked ones (13). One approach to identify targets is therefore to scan the genome for regions of high diversity or other related features, such as intermediate allele frequencies (14). A challenge is that such

patterns of diversity can occur by chance because of the tremendous variance in coalescence times due to genetic drift alone (14). As an illustration, under a simple demographic model with no selection, the probability that two human lineages do not coalesce before the split with chimpanzee is on the order of 10^{-4} (15, 16). Although this probability is small, the human genome is large, and so many such regions could occur by chance. To circumvent this difficulty, we looked for cases in which an ancestral polymorphism has persisted to the present time in both humans and chimpanzees, that is, is shared identically by descent between the two species. This outcome is not expected to occur by genetic drift alone because it requires that neither human nor chimpanzee lineages coalesce before the human-chimpanzee ancestor, which is unlikely even in a large genome (16).

To date, two cases of human polymorphisms shared with other apes have been shown to be identical by descent [additional background is available in fig. S1 and (16)]: variants in the major histocompatibility complex (MHC), a complex encoding cell surface glycoproteins that present peptides to T cells (17), and polymorphisms at ABO, a glycosyltransferase, that underlie the A and B blood groups (18). Ancient balancing selection leaves a narrow footprint in genetic variation (15, 18), however, which may be particularly difficult to detect without dense variation data (19). Thus, the recent availability of genome sequences for multiple humans and chimpanzees provides an opportunity to search comprehensively and with greater power for ancient balancing selection.

Identification of shared SNPs and haplotypes.

We examined complete genome sequences from 59 humans from sub-Saharan Africa (Yoruba) (20) and 10 Western chimpanzees (*Pan troglodytes verus*) (21) in order to identify shared polymorphisms—namely, high-quality orthologous SNPs with identical alleles in the two species (table S1) (16). In total, 33,906 autosomal and 492 X-linked single-nucleotide polymorphisms (SNPs) passed our filters (table S2). The lower proportion of shared SNPs found on the X (in humans, 0.36% of autosomal SNPs versus 0.19% of X-linked

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SNPs) is expected under neutrality because of the lower mutation rate and the smaller effective population size of the X (22).

The set of shared SNPs has similar properties to those of nonshared SNPs in terms of mapping quality, depth of coverage, and proportion in repeats (fig. S2 and table S2), which is consistent with it containing few artifacts. The shared SNPs include a much higher proportion of CpGs, however: 71.5% of autosomal shared SNPs occur at CpG dinucleotides, whereas only 26.4% of all human SNPs have this property (table S2). Because CpGs are known to have a higher mutation rate than other sites (23), this observation, along with the similarity in allele frequency distributions of shared and nonshared SNPs (fig. S2), suggest that most instances of shared SNPs are due to the independent occurrence of the same mutation in both species—in other words, that most SNPs are identical by state rather than descent (16).

Nonetheless, SNPs are shared between humans and chimpanzees 1.3-fold more often than is expected by chance, after controlling for the composition of the adjacent base pairs [the sequence context thought to have the strongest effect on mutation rate variation (23)] (fig. S3). This excess may be explained by residual effects on the mutation rate of the sequence context beyond the adjacent base pairs (fig. S4) or by variation in selective constraint across sites, but could also reflect instances of balancing selection.

Within the set of shared SNPs, we sought to enrich for targets of balancing selection by two approaches (Fig. 1A). First, we considered shared coding SNPs (16), a set that a priori should contain more functional changes subject to purifying selection so is less likely to include polymorphisms shared by chance alone. Second, to home in on cases with unequivocal evidence for balancing selection, we searched for polymorphisms shared because of identity by descent. Where

balancing selection acted on a single site and maintained a polymorphism stably since the human-chimpanzee split, a short ancestral segment should persist until the present around the selected site, of expected length less than 4 kilobases (kb) [depending on the recombination rate (16)]. This segment is likely to contain one or more neutral, shared polymorphisms that arose in the ancestral population of humans and chimpanzees and are in strong or complete linkage disequilibrium (LD) with the selected site (Fig. 1B) (15, 18). Thus, this scenario should produce specific patterns of haplotype sharing between species. Guided by these considerations, we focused on cases with two or more shared SNPs within 4 kb and in significant LD in humans and in chimpanzees, with the same coupling of alleles in the two species (henceforth “shared haplotypes”) (16). These LD criteria should almost always be met when a neutral polymorphism has persisted because of close linkage with an ancient balanced polymorphism and yet are expected to filter out the vast majority (>96%) of cases of neutral, recurrent mutations (table S3) (16). These LD criteria should also be met if balancing selection acted on two or more sites and there is epistasis between them (as is the case at *ABO*), in which case the shared haplotypes may be longer (Fig. 1B).

We imposed stringent quality control filters on the shared haplotypes and coding SNPs (Fig. 1A) in order to exclude regions with highly similar paralogs present in the reference genomes of humans or chimpanzees as well as artifacts arising from duplicates that are either fixed or polymorphic in the two species but for which one copy is absent from both reference genomes (these filters should also weed out regions that experience paralogous gene conversion) (16). After filtering, we combined regions with shared haplotypes if they had a shared SNP in common (tables S4 and S5).

Protein variants. Across the genome, the MHC stood out (fig. S5), with 11 shared nonsynonymous and seven shared synonymous SNPs, including six nonsynonymous and three synonymous that were not among the many cases of shared haplotypes in this region (table S6) (16).

Unexpectedly, given that the basis for A and B blood groups is shared between humans and gibbons but not chimpanzees (who lack the B type) (18), we found two SNPs shared between humans and chimpanzees in *ABO* ~4 kb from the sites that distinguish A and B blood types in humans (fig. S6). Neither shared SNP is nonsynonymous (one is synonymous, the other intronic), and they do not meet our criteria for creating shared haplotypes, but there is a peak of diversity around them within both humans and chimpanzees, suggesting that they may be ancient variants (fig. S6).

In addition, we found 199 synonymous SNPs, 135 nonsynonymous SNPs, and 1 premature stop shared between humans and chimpanzees, distributed among 324 genes (table S5). Notable among these is a nonsynonymous SNP in *GP1BA*, a gene encoding a glycoprotein present on the membrane of platelets that is responsible for binding to the ABO antigens expressed on the Von Willebrand Factor (VWF) (24). The specific polymorphism in *GP1BA* shared between humans and chimpanzees, corresponding to the human platelet alloantigen 2 (HPA-2) polymorphism, affects the binding affinity to VWF and is associated with platelet count (25). More generally, the blood glycoprotein VWF is used as a bridge to anchor platelets to injured blood vessels for coagulation, and variants in *ABO* are strongly associated with protein levels of VWF (24). These findings suggest that two genes associated with the same complex may have been targets of long-lived balancing selection.

Regions with shared haplotypes. We identified 125 regions outside the MHC with shared

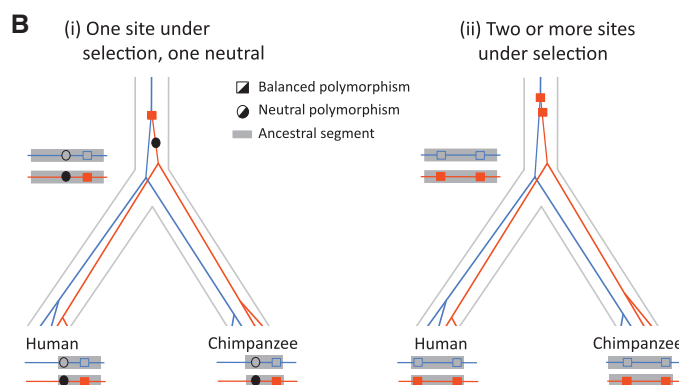
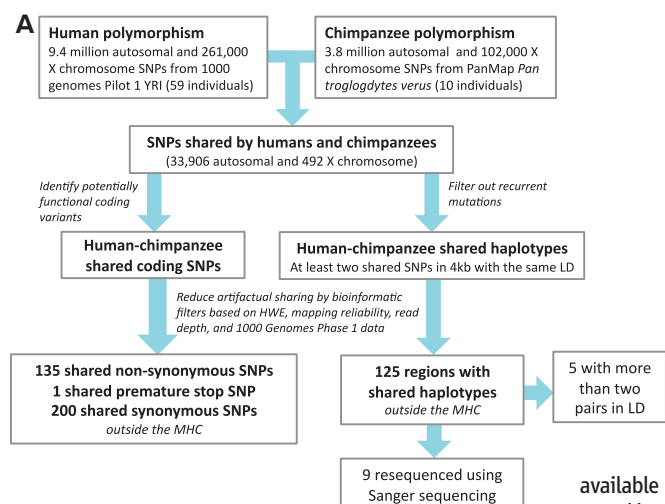


Fig. 1. Analysis pipeline. (A) Diagram of the pipeline to identify shared coding SNPs and shared haplotypes. Details of the filtering and validation are available in (16). (B) Two possible scenarios of ancient balancing selection that may be detected by our approach. In (i), only one site is under balancing selection, and a second mutation is maintained by balancing selection because of tight linkage to the selected site. In (ii), two or more epistatically interacting polymorphic sites are maintained by balancing selection from the ancestral population of human and chimpanzee to the present time. In this case, the ancestral segment could be substantially longer because there is selection against recombinant haplotypes.

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haplotypes between humans and chimpanzees, whose total lengths span 4 bp to 6649 bp (table S4). In five of the regions (nearest *FREM3*, *MTRR*, and *PROKR2* and in *HUS1* and *IGFBP7*), there are more than two pairs of shared SNPs in significant LD, which simulations suggest should never occur in the genome by neutral recurrent mutations alone (16).

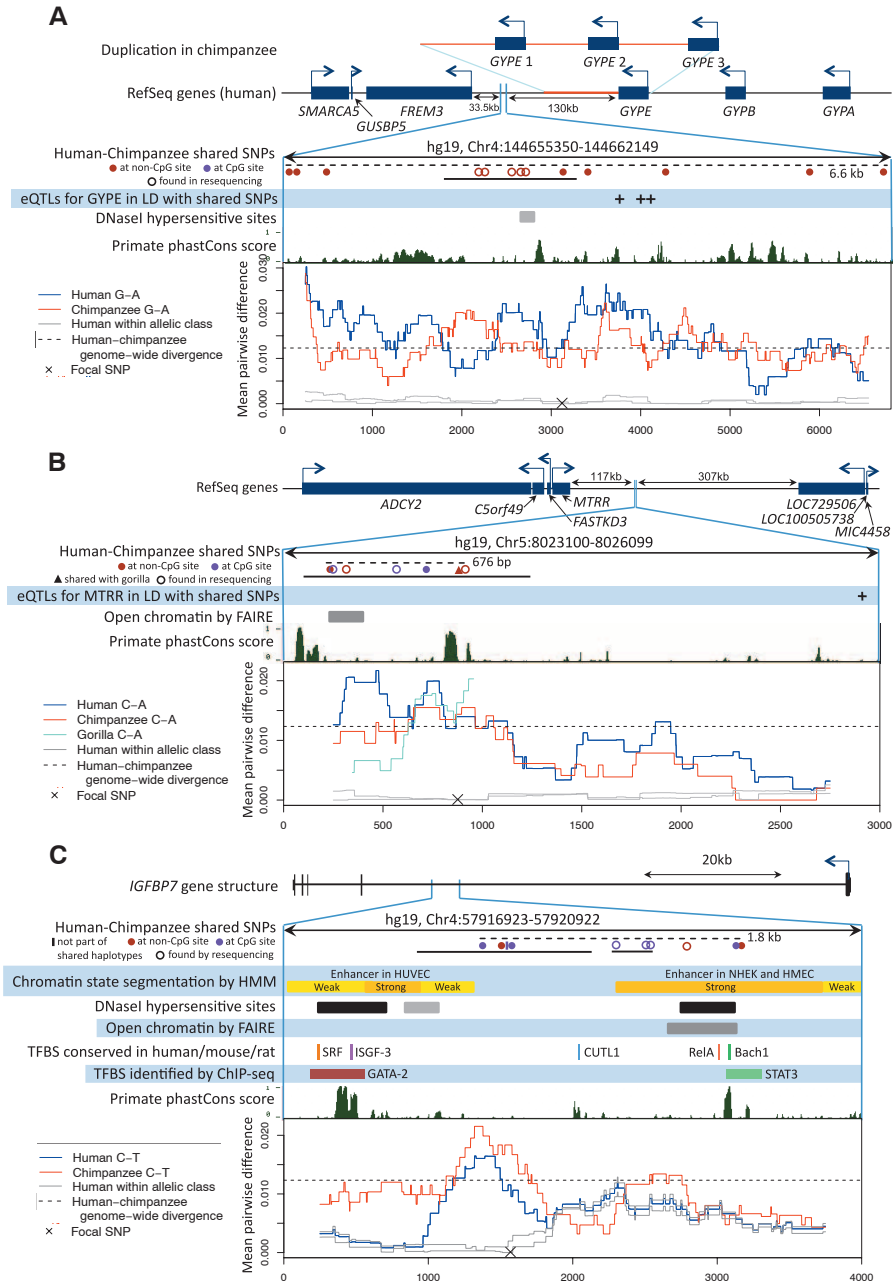
In the regions nearest *FREM3* and *MTRR* and in *IGFBP7*, there is a peak of diversity in humans and chimpanzees around the shared SNPs that is comparable with or in excess of the average divergence between the two species (and yet there is no evidence for elevated mutation rates in the region, as assessed by the levels of divergence between more distant out-group species), which is consistent with the

polymorphisms predating the human-chimpanzee split (Fig. 2 and fig. S7). Furthermore, when we built a phylogenetic tree based on these regions, haplotypes from different species that carry the same allele are more closely related to each other than to haplotypes from the same species with the other allele (with high posterior probability and based on 800 bp or more) (Fig. 3, A to C) (16). This clustering pattern establishes that these cases cannot be explained solely by recurrent mutation (16).

The shared SNPs nearest *FREM3* are in almost perfect LD with several expression quantitative trait loci (eQTLs) for *GYPE* (~130 kb away) in monocytes (Fig. 2A). Along with *GYPB* and *GYPB*, *GYPE* originated from one copy in the common ancestor of African apes (26). *GYPB*

is a known receptor for *Plasmodium falciparum* proposed to be under balancing selection in humans, which, together with *GYPB*, codes for the MNS blood group (26); much less is known about *GYPE*, but it may also specify the M blood group antigen (27). The shared SNPs ~117 kb from *MTRR*, a gene involved in the production of methionine and implicated in the regulation of folate metabolism, are also in significant LD with an eQTL in monocytes, for *MTRR* (Fig. 2B). In turn, the shared SNPs in an intron of *IGFBP7* occur in a likely enhancer (Fig. 2C). *IGFBP7* has been shown to regulate cell proliferation, cell adhesion, and angiogenesis in cancer cell lines and plays a role in innate immunity by interacting with chemokines implicated in the regulation of lymphocyte trafficking (28).

Fig. 2. Functional information for three regions with a polymorphism shared identical by descent in humans and chimpanzees. We show the nearby genes and direction of transcription, then a close up of the region with shared polymorphisms between humans and chimpanzees. The original shared SNPs used to identify shared haplotypes are shown as solid circles. The region resequenced in the validation experiment is indicated with a solid black bar, and the length of the shared haplotypes is indicated with a dashed black bar (16). Sources of the functional annotation tracks shown are available in (16); darker shading for the formaldehyde-assisted isolation of regulatory elements (FAIRE) and DNaseI hypersensitivity tracks indicates a more intense signal. In the bottom panels, we focus on a shared SNP (hg19, chr4:144658471, chr5:8023976, and chr4:57918492, respectively) and show the mean pairwise difference between allelic classes for humans (in blue) and chimpanzees (in red), for a 500-bp sliding window; the mean pairwise difference within an allelic class in humans is in gray. We further indicate the average genome-wide divergence between human and chimpanzee (1.2%) (39) with a dotted black line. Divergence between more distant ape species and a zoom out of diversity levels in each region can be seen in figs. S7 and S9. (A) *FREM3*. A duplication in chimpanzees that includes the *GYPE* gene is shown above the gene structure in humans (26). The shared SNPs and eQTLs for *GYPE* in monocytes (40) are in almost perfect LD, with a pairwise correlation coefficient (r^2) ranging from 0.98 to 1. (B) *MTRR*. The shared SNP represented by a triangle is also seen in a sample of seven gorillas by Sanger resequencing (16); pairwise differences between allelic classes in gorillas is shown in turquoise for the resequenced region. The maximum pairwise r^2 between a shared SNP and the eQTL for *MTRR* in monocytes is 0.47 (16, 40). The FAIRE signal is enriched in six cell lines. (C) *IGFBP7*. In the scan for shared haplotypes, five shared SNPs were found within 4 kb, occurring in two clusters with three and two SNPs, respectively, which are not in LD with each other in humans. Two of the shared SNPs found in the resequencing and a SNP outside the resequenced region constitute an additional instance of shared haplotypes. The FAIRE signal is enriched in four cell lines. Using a focal SNP in the second cluster yields similar results (fig. S7).



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In the two other regions (in *HUS1* and nearest *PROKR2*) as well as in a region with only one pair of shared SNPs in significant LD (nearest *ST3GAL1*), diversity levels are only unusually high in humans, but nonetheless a phylogenetic tree for a small subset of the region (300 bp) clusters by allele and not by species (Fig. 3, D to F, and fig. S8). These patterns are consistent with the presence of an ancient balanced polymorphism on an ancestral segment that has been highly eroded by recombination [a more in-depth discussion is available in (16)]. *PROKR2* is a

receptor that functions as a proinflammatory mediator and whose ligand is able to modulate immune response (29). In turn, *ST3GAL1* is a sialyltransferase that modifies the cell-surface glycan structure of dendritic cells (30) and for which knockout mice lack peripheral CD8⁺ T lymphocytes (31).

To check for possible sequencing or mapping errors, we resequenced the six regions with evidence for a polymorphism shared identical by descent (summarized in table S7) in 11 to 12 humans, 10 to 12 chimpanzees, and four to seven gorillas.

In all cases, we confirmed the presence of the expected shared SNPs and the predicted LD patterns among them (16). Additionally, we found that in the *MTRR* and *ST3GAL1* regions, one of the SNPs in the shared haplotypes is also segregating in gorilla (Fig. 2B and fig. S8) (16).

Common properties of ancient balanced polymorphisms. The narrow signature of ancient balancing selection allows the possible causal sites to be delimited to a few kilobases. Of the six regions with evidence for a long-lived balanced polymorphism, those in *HUS1* and *IGFBP7* and nearest *ST3GAL1* likely have regulatory activity (Fig. 2 and fig. S8). More generally, only two of the 125 candidate regions include a shared SNP that is coding (in both cases, synonymous), but at least 10 regions appear to have a regulatory role (table S8) (16). Our findings therefore suggest that balancing selection has targeted regulatory variation in the human genome. The possible mechanisms underlying the maintenance of such polymorphisms are unclear but could involve allele-specific properties that lead to differences in levels of expression, in response to stimuli, or in patterns of expression across tissues [as is the case for *B4galnt2* in mice (32)].

To further assess the commonalities among the set of 125 regions, we tested for an enrichment of gene categories for the nearest protein-coding gene (Table 1 and table S9) (16). We found significant enrichments of a number of overlapping categories, driven by the presence of 24 membrane glycoproteins in the test set of 54 genes ($P < 10^{-3}$, corresponding to a 2.4-fold enrichment of glycoproteins over the background and a 1.2-fold enrichment of membrane glycoproteins over a background of only glycoproteins) (Table 1 and tables S10 to S12). Five of the 24 membrane glycoproteins have an immunoglobulin I-set domain ($P = 0.006$; a 6.3-fold enrichment over a background of membrane glycoproteins). The same trends are seen when considering an almost completely independent set of 335 coding SNPs (only two occur in shared haplotypes, neither of which contributes to these trends): Glycoprotein and cell adhesion are top categories among shared coding SNPs ($P < 0.02$) (tables S13 and S14). Although the number of genes involved is small, there is also an enrichment of gene ontology categories related to galactosyltransferase activity among genes near shared

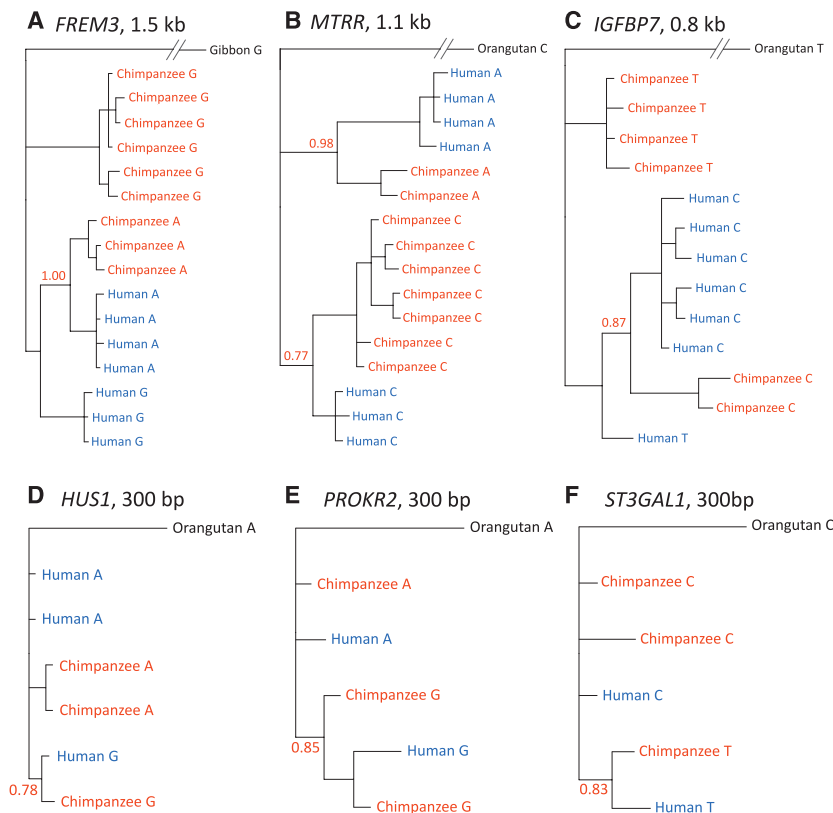


Fig. 3. Phylogenetic trees of haplotypes labeled with the same focal SNP considered in Fig. 2 or fig. S8 for (A) *FREM3*, (B) *MTRR*, (C) *IGFBP7*, (D) *HUS1*, (E) *PROKR2*, and (F) *ST3GAL1*. Trees were generated from our resequencing data by using MrBayes, with the median posterior probability of the clade over two runs reported in red (16). Results are for the entire resequenced regions for *FREM3* and *MTRR*, and for the largest regions for which we found strong support in other cases. For *FREM3*, *MTRR*, and *IGFBP7*, the regions on which the trees are based are long (>800 bp), providing strong support for a polymorphism shared identical by descent (16). For *HUS1*, the tree still clusters by allele when considering 1 kb (with posterior probability 0.58), but for *ST3GAL1* and *PROKR2*, this is not the case [more details are available in (16)].

Table 1. Enrichment analysis. Gene category enrichment of the closest gene within 20 kb of shared human-chimpanzee haplotypes (16). We show only the top categories, for which $P < 10^{-3}$ (a longer list is available in table S9); because the categories overlap, the Bonferroni correction is conservative. “Count” refers to the number of genes from the gene set with

the given property (Term); “List total” refers to number of genes from the gene set that can be annotated in the category; “Pop hits” refers to the number of genes in the background with the given property; and “Pop total” refers to the number of genes from the background that can be annotated in the category.

Category	Term	Count	List total	Pop hits	Pop total	Fold enrichment	P value	Bonferroni corrected P value
SP_PIR_KEYWORDS	Glycoprotein	27	50	3733	16731	2.42	2.56×10^{-6}	3.33×10^{-4}
GOTERM_CC_FAT	GO:0031224~intrinsic to membrane	31	42	4719	11298	1.77	4.48×10^{-5}	0.0051
INTERPRO	IPR013098:Immunoglobulin I-set	6	46	133	14738	14.45	5.08×10^{-5}	0.0076
INTERPRO	IPR007110:Immunoglobulin-like	7	46	373	14738	6.01	8.93×10^{-4}	0.1254

haplotypes and for categories related to glycosylation among genes with a shared coding SNP (tables S9 and S14).

Given that viruses frequently use host glycans to gain entry into host cells and some bacteria imitate host glycans to evade the host immune system (33–35), these enrichments suggest that the targets of balancing selection that we identified likely evolved in response to pressures exerted by human and chimpanzee pathogens, mirroring what is known about other genes under balancing selection in humans [(1, 17, 18, 36) and references therein]. Moreover, the observation that variation at loci that lie at the interface of host-pathogen interactions was stably maintained for millions of years is consistent with the hypothesis that arms races between hosts and pathogens can result not only in transient polymorphisms but also, in the presence of a cost to resistance, to a stable limit cycle in allele frequencies in the host (4, 9, 37).

We found several instances of ancient balancing selection in humans in addition to the two previously known cases. Our analysis suggests that this mode of selection has not only involved protein changes but also the regulation of genes involved in the interactions of humans and chimpanzees with pathogens and points to membrane glycoproteins as frequent targets. Because we deliberately focused on the subset of cases of balancing selection that are the least equivocal—requiring variation at two or more sites to be stably maintained in the two species from their split to the present—we likely missed balanced polymorphisms with a high mutation rate to new selected alleles [that is, with high allelic turnover (38)], in which the ancestral segment has been too heavily eroded by recombination, as well as any instance in which balancing selection pres-

ures are more recent than the human-chimpanzee split. Thus, it seems likely that many more cases of balancing selection in the human genome remain to be found.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S9
Tables S1 to S20
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REPORTS

Topology-Driven Magnetic Quantum Phase Transition in Topological Insulators

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The breaking of time reversal symmetry in topological insulators may create previously unknown quantum effects. We observed a magnetic quantum phase transition in Cr-doped Bi₂(Se_xTe_{1-x})₃ topological insulator films grown by means of molecular beam epitaxy. Across the critical point, a topological quantum phase transition is revealed through both angle-resolved photoemission measurements and density functional theory calculations. We present strong evidence that the bulk band topology is the fundamental driving force for the magnetic quantum phase transition. The tunable topological and magnetic properties in this system are well suited for realizing the exotic topological quantum phenomena in magnetic topological insulators.

The metallic surface states of three-dimensional (3D) topological insulators (TIs) are protected by time reversal symmetry (TRS)

(1–3). Although breaking the TRS is generally detrimental to these states, it may also lead to exotic topological quantum effects. Examples

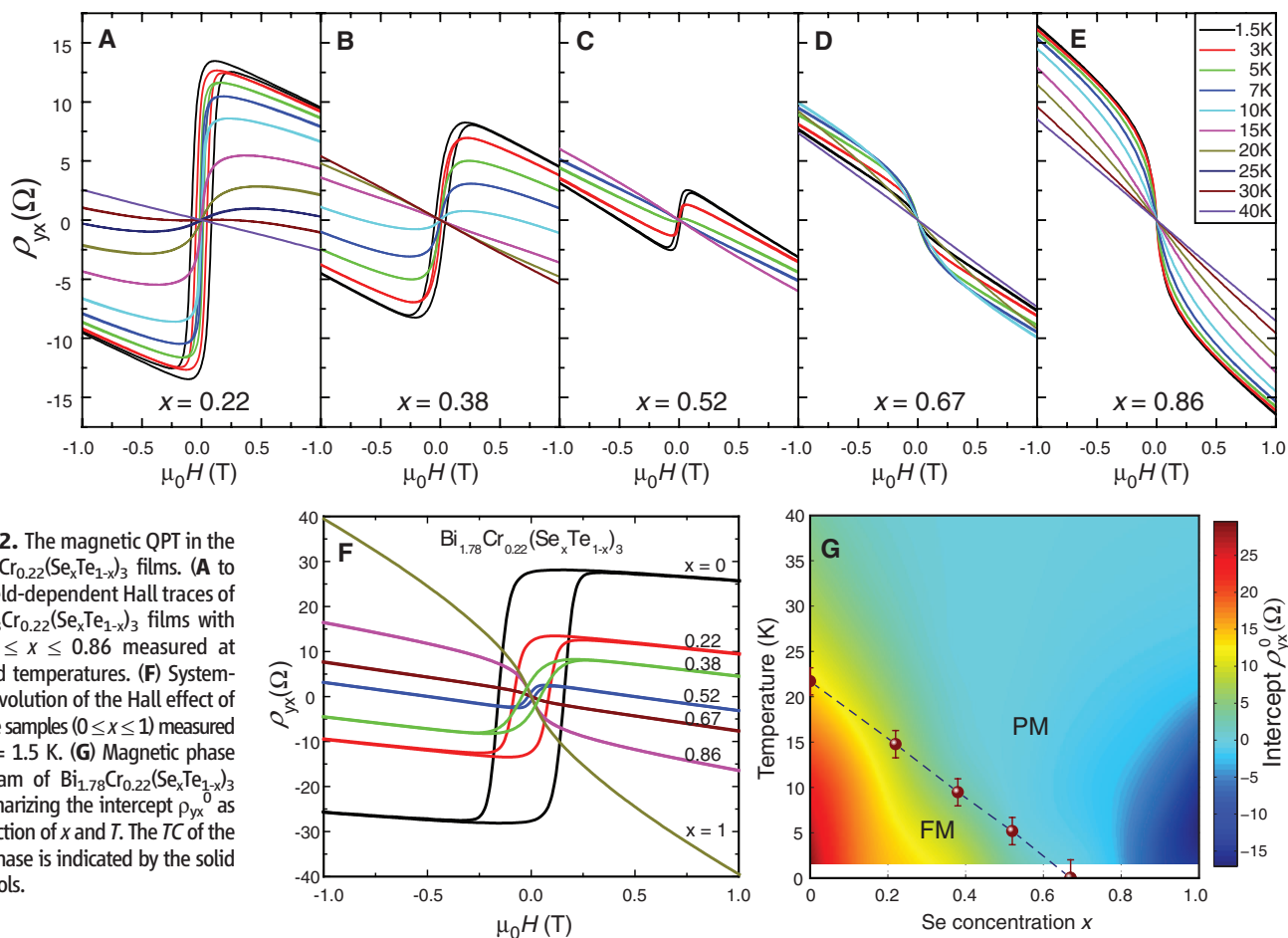
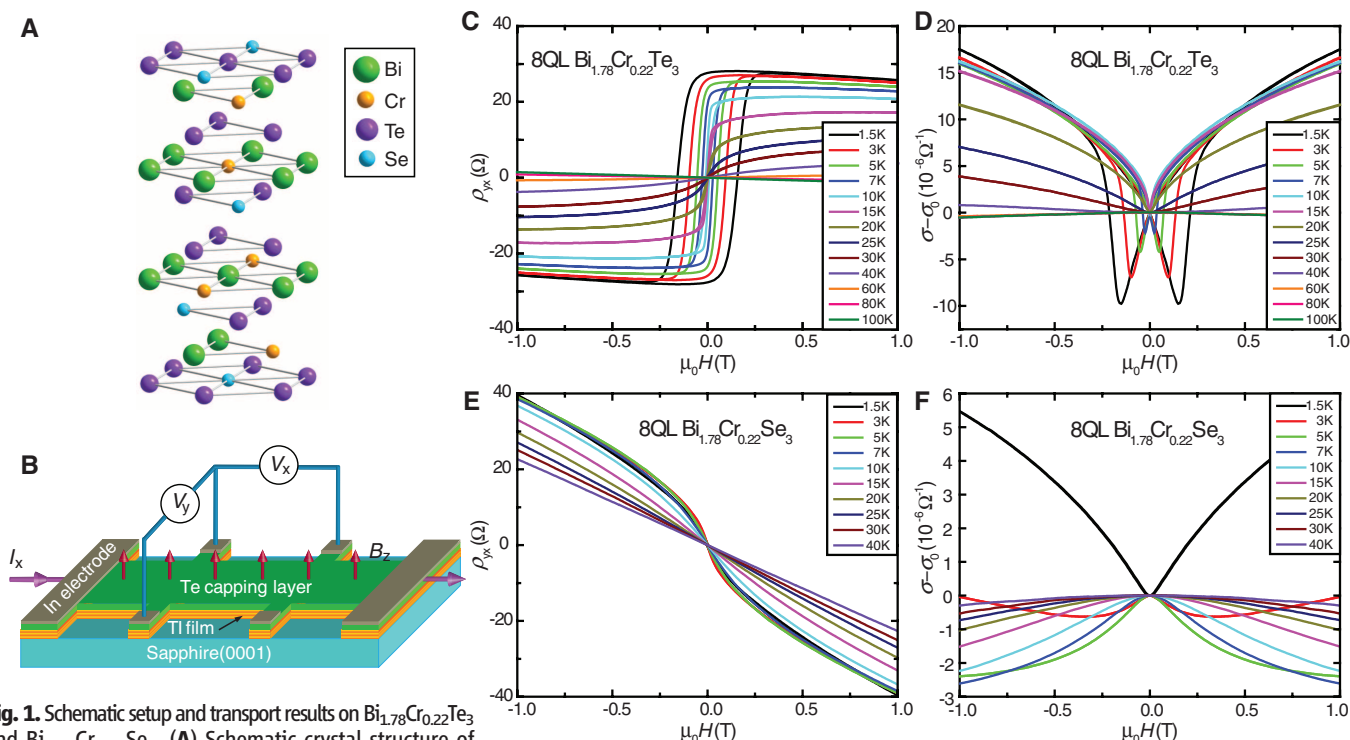
include image magnetic monopoles (4), a quantized anomalous Hall effect (5, 6), giant magnetooptical effects (7), and a dissipationless inverse spin-Galvanic effect (8). A key step for realizing these previously unknown quantum states is to tune the magnetic ordering in TIs in a controlled manner and investigate the interplay between magnetism and topological order.

With their large bulk gap and a single-surface Dirac cone, Bi₂Te₃ and Bi₂Se₃ (9–11) are widely used as hosts for TRS-breaking perturbations. In Mn-doped Bi₂Te₃ single crystals, magnetization

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measurements demonstrate a ferromagnetic (FM) state with a Curie temperature (T_C) of up to 12 K (12). On the cleaved surface of an Fe-doped Bi_2Se_3 single crystal, angle-resolved photoemission spectroscopy (ARPES) reveals the opening of an energy gap at the Dirac point (13). Iron (Fe) atoms deposited on the surface of a Bi_2Se_3 single crystal are found to create odd multiples of Dirac-like surface states (14). Scanning tunneling microscopy on the surface of magnetically doped TI crystals demonstrates that breaking TRS can lead to previously unknown quasiparticle interference patterns (15) and strong spatial variations for the helical surface states (16).

Most of these previous studies focused on the effect of magnetism on the topological surface states; however, little is known about how the magnetic ordering is affected by the topological property. Because the Z_2 bulk topology is the most fundamental identity of a TI, it probably also plays a role in determining the phases and phase transitions in magnetically doped TIs. To test this conjecture, we fabricated chromium (Cr)-doped $\text{Bi}_2(\text{Se}_x\text{Te}_{1-x})_3$ TI films using molecular beam epitaxy (17). By varying the mixing ratio of Bi_2Se_3 and Bi_2Te_3 , we can actively modify

the strength of spin-orbit coupling (SOC), which is essential for the band inversion of TIs. In the resulting $\text{Bi}_{2-y}\text{Cr}_y(\text{Se}_x\text{Te}_{1-x})_3$ (Fig. 1A), the Cr dopants substitute Bi sites, and Se/Te atoms are randomly mixed. All of the films have the same thickness $d = 8$ quintuple layers (QLs), so that they are in the 3D regime with decoupled surfaces (18). The Cr content is fixed at $y = 0.22$ because at this doping level, the density of local moments is high enough to sustain long-range magnetic order, and the SOC strength is reduced to the verge of a topological phase transition (19).

Magneto transport measurements on the TI films (Fig. 1B) are made in the presence of an external magnetic field (H) perpendicular to the film plane. At the base temperature $T = 1.5$ K, the Hall effect of the 8-QL $\text{Bi}_{1.78}\text{Cr}_{0.22}\text{Te}_3$ film (Fig. 1C) shows a hysteretic loop and a nearly square-shaped positive jump, the hallmarks of anomalous Hall effect (AHE) in FM conductors (20). The total 2D Hall resistivity ρ_{yx} can be expressed as $\rho_{yx} = R_A M(T, H) + R_N H$, where $M(T, H)$ is the magnetization and R_A and R_N are the anomalous and normal Hall coefficients, respectively. Both the anomalous Hall resistivity and the coercive force (H_{coer}) decrease as T rises.

The Hall traces become fully reversible at $T > 20$ K; thus, the T_C of this film is around 20 K. The normal Hall effect at high H has a negative slope for the entire temperature range (fig. S2), indicating the existence of electron-type charge carriers. The magnetoconductivity (MC) curves taken at $T < T_C$ show butterfly-shaped hysteresis at weak H (Fig. 1D), as commonly observed in FM metals. MC keeps increasing at higher H , which is indicative of the weak localization (WL) of charge carriers instead of the weak antilocalization (WAL) in pristine Bi_2Te_3 (21).

The Cr-doped Bi_2Se_3 exhibits a very different transport behavior. At $T = 1.5$ K, the Hall trace of $\text{Bi}_{1.78}\text{Cr}_{0.22}\text{Se}_3$ film (Fig. 1E) has a pronounced negative curvature at weak H but shows no observable hysteresis, which is consistent with the field-induced AHE in paramagnetic (PM) materials without spontaneous magnetization. The MC curves also show no sign of hysteresis even at the base temperature (Fig. 1F). MC changes from positive at low T to negative at high T , which is consistent with the WL-to-WAL crossover reported previously (22). The possible existence of in-plane ferromagnetism in the $\text{Bi}_{1.78}\text{Cr}_{0.22}\text{Se}_3$ film is ruled out by magnetization and magneto transport

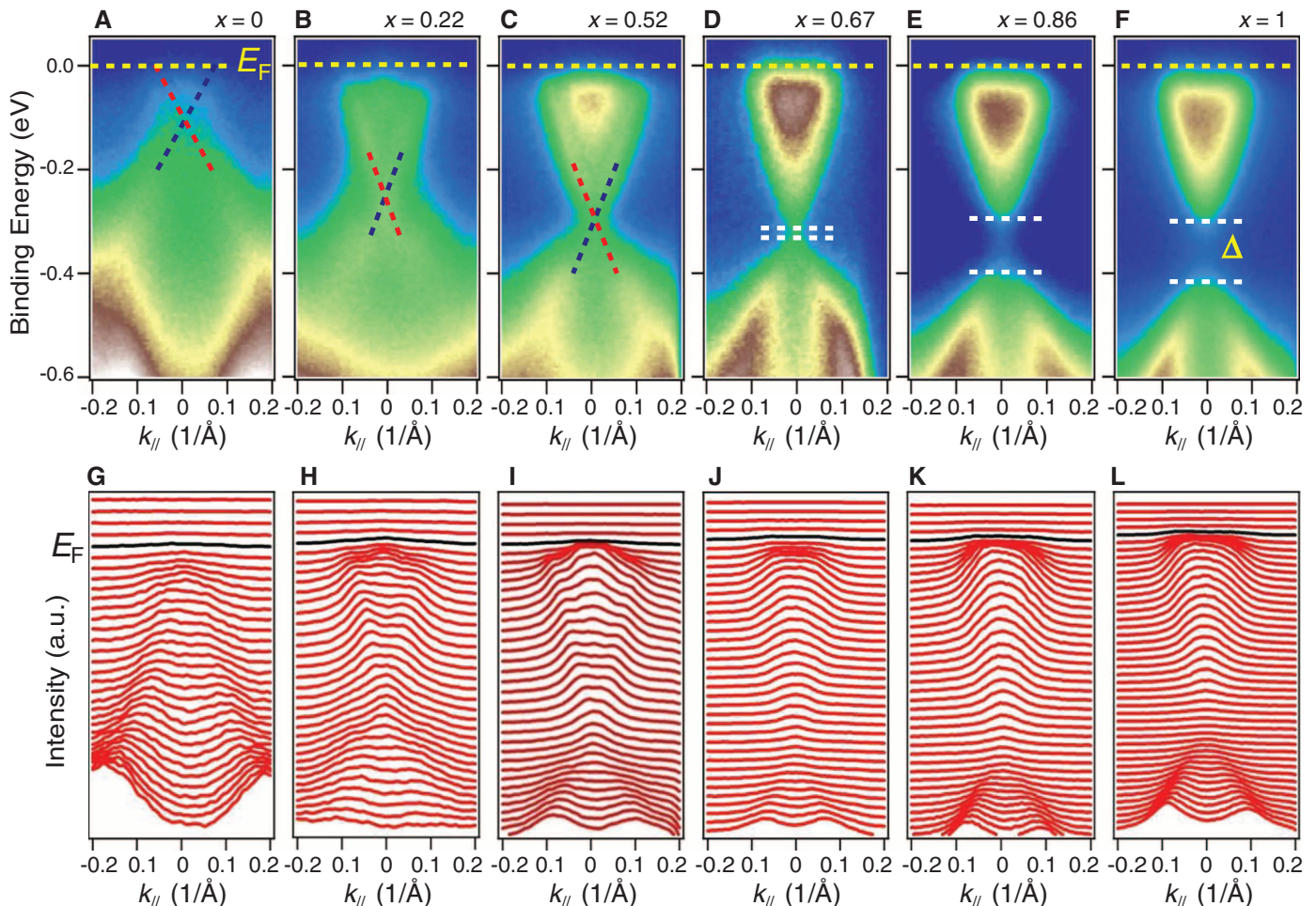


Fig. 3. ARPES measurements on $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$ films. (A to F) ARPES band maps and (G to L) the MDCs taken at 120 K along the K- Γ -K direction on $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$, with $x = 0, 0.22, 0.52, 0.67, 0.86$ and 1.0 . The blue and

red dashed lines in (A) to (C) indicate the surface states with opposite spin polarities. In (D) to (F), an energy gap Δ opens between the bulk valence band and conduction band.

measurements with H applied along the film plane (fig. S12).

To uncover the origin of the sharp contrast between the magneto transport properties of Cr-doped Bi_2Te_2 and Bi_2Se_3 , we fabricated Cr-doped $\text{Bi}_2(\text{Se}_x\text{Te}_{1-x})_3$, an isostructural, isovalent mixture of Bi_2Te_3 and Bi_2Se_3 . The Hall traces are displayed in Fig. 2, A to E, measured on five $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$ films with $0.22 \leq x \leq 0.86$. The three Te-rich samples with $x \leq 0.52$ all show a hysteretic response at low T , reflecting FM ordering. As the Se content is increased to $x = 0.67$, however, the hysteresis disappears, and the system becomes PM. The sign of the AHE also reverses to negative right at this doping. With further increase of x to 0.86, the system remains PM, and the negative AHE becomes more pronounced. The FM-to-PM phase transition can also be seen in the MC curves (fig. S4) (17).

The Hall curves of all the $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$ films measured at $T = 1.5$ K are summarized in Fig. 2F, revealing a highly systematic evolution of H_{coer} and the intercept Hall resistivity ρ_{yx}^0 (fig. S6A). Because the magnetism of the system can be characterized by the AHE, we can con-

struct a magnetic phase diagram by plotting the ρ_{yx}^0 values of each sample and at each T (Fig. 2G). At the base temperature, the phase diagram is separated into two distinct regimes: an FM phase with positive ρ_{yx}^0 and a PM phase with negative ρ_{yx}^0 . Because the transition between the two magnetic phases occurs at the ground state, it is a quantum phase transition (QPT) driven by the change of chemical composition. The quantum critical point (QCP) $x_c \sim 0.63$ can be estimated from the interpolated x value when ρ_{yx}^0 changes sign (fig. S6B). The solid symbols in Fig. 2G indicate the T_c of each sample determined by the temperature when H_{coer} becomes zero.

ARPES measurements on $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$ uncover a surprising feature. The ARPES band maps displayed in Fig. 3A were taken at $T = 120$ K, when all the samples are in the PM state. The Fermi level of all the samples lies above the Dirac point, which is consistent with the negative slope of the normal Hall effect. The three samples with $x \leq 0.52$ show well-defined gapless surface states with linear dispersions. The surface-state features can be better identified by the dual-peak structures around the Γ point in the momentum

distribution curves (MDCs) (Fig. 3, G to I). As x is increased to $x = 0.67$ (Fig. 3D), however, the surface state features can no longer be resolved, and a small energy gap starts to appear at the Γ point of the band structure. With further increase of x up to $x = 1$, the surface states are always absent, whereas the gap amplitude keeps increasing. Because the surface states derive from the nontrivial bulk topology, their absence for $x \geq 0.67$ suggests that the bulk band structure in this regime is topologically trivial. Therefore, the ARPES results reveal a topological QPT, a transition from the TI to trivial band insulator, accompanying the magnetic QPT. The ARPES patterns are very similar to that in $\text{BiTi}(\text{S}_{1-\delta}\text{Se}_\delta)_2$ TIs, showing the topological QPT induced by S substitution of Se (23, 24).

The topological QPT is also corroborated through density functional theory (DFT) calculations (17). The signature of a topological QPT in the bulk band structure, a gap closing point at the critical SOC strength, can be seen clearly in the DFT results on Cr-doped Bi_2Se_3 (fig. S7). The topological QPT is caused by the reduced SOC strength resulting from the Cr substitution

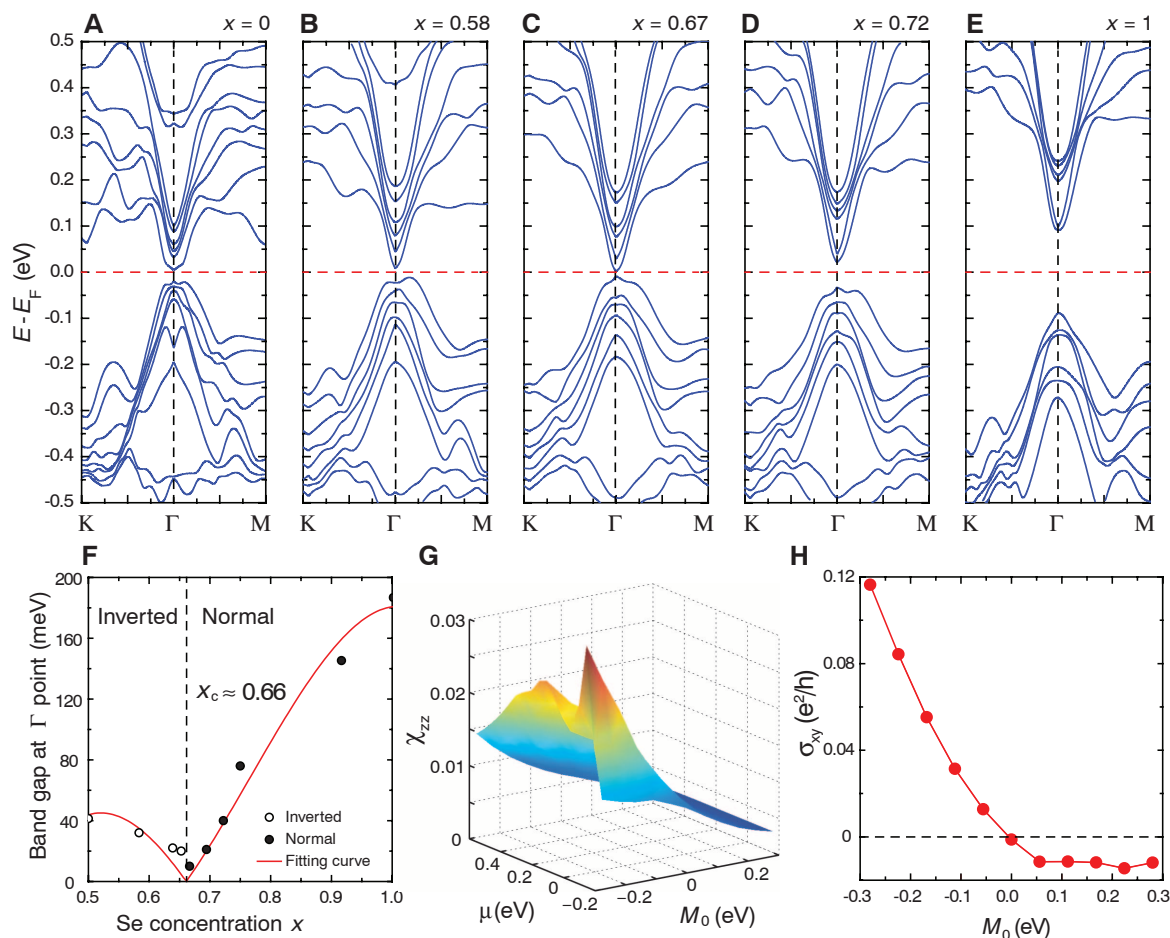


Fig. 4. Theoretical calculations of the band structure and magnetic properties. DFT-calculated bulk band structure of $\text{Bi}_{1.75}\text{Cr}_{0.25}(\text{Se}_x\text{Te}_{1-x})_3$, with (A) $x = 0$, (B) 0.58, (C) 0.67, (D) 0.72, and (E) 1.0. (F) The topological phase diagram. Between $x = 0.5$ and critical point $x_c = 0.66$, the bulk band structure is inverted.

Above that, the band structure becomes normal. (G) The calculated spin susceptibility of the four-band model for different μ and M_0 . (H) Anomalous Hall conductivity σ_{xy} as a function of M_0 with $G_{z1} = 0$ and $G_{z2} = 0.02$ eV at fixed $\mu = 0.4$ eV showing the sign reversal of σ_{xy} across the topological QPT.

of Bi. At sufficiently high Cr doping, the SOC is not strong enough to invert the bands, leading to a trivial bulk topology (19). In contrast, for Cr-doped Bi_2Te_3 our DFT calculations show that the bulk band remains inverted for Cr content up to $y = 0.25$ (figs. S8 and S9). The more robust band inversion is a result of the larger SOC strength of Te as compared with Se. The calculated band structures of $\text{Bi}_{1.75}\text{Cr}_{0.25}(\text{Se}_x\text{Te}_{1-x})_3$ with varied x (Fig. 4, A to E) show a transition from inverted to normal bands caused by the reduced SOC strength with increasing Se/Te ratio. The calculated bulk gap at the Γ point is summarized in Fig. 4F, which clearly shows a topological QPT near $x \sim 0.66$, which is in agreement with the experiments.

With the correlation between the magnetic and topological QPTs firmly established, we turned to a more fundamental question: Which phase transition is the driving force, and which one is the consequence? Two pieces of evidence support the scenario that topology determines the magnetic ordering. First, in our $\text{Bi}_{1.78}\text{Cr}_{0.22}(\text{Se}_x\text{Te}_{1-x})_3$ samples the Cr content is fixed, and only the Se/Te ratio is varied. This provides a knob for fine-tuning the SOC strength—hence, the bulk band topology—but the magnetic property is not directly affected. Therefore, the magnetic QPT should be a secondary effect of the topological QPT. Second, the ARPES results show that even at high T when all the samples are in the PM state, the two regimes separated by the QCP already develop different topologies. At low T , the magnetic ground states form following the preformed topological character, with the FM phase resulting from the nontrivial topology and transitioning to the PM phase when the bulk turns topologically trivial.

The topological origin of the magnetic QPT is further supported by the effective model calculations (17). We calculated the z -direction spin susceptibility (χ_{zz}) of eight QL magnetically doped TI films using an effective four-band model (Fig. 4G) as a function of the chemical potential (μ) and the mass term (M_0). In the inverted regime with $M_0 < 0$, χ_{zz} always remains a large value when μ is around the gap, as a consequence of the van Vleck mechanism (5); the second-order matrix element is strongly enhanced when the bulk bands become inverted. The topologically nontrivial phase thus strongly favors an FM ordering, which naturally explains the topology-driven magnetic QPT discovered in the experiments. The van Vleck mechanism is further supported by the magnetization measurements (fig. S10), which show that the ferromagnetism occurs in the bulk rather than on the surfaces (25, 26). The out-of-plane magnetic anisotropy (fig. S11) is also consistent with the van Vleck-type FM order in TIs (5).

To reveal the physical origin of the AHE sign change at the QCP, we calculated σ_{xy} based on the four-band model, with two additional Zeeman splitting terms, G_{z1} and G_{z2} , from the exchange coupling between the electrons and magnetic impurities (17). The σ_{xy} value is summarized in

Fig. 4H as a function of M_0 with fixed chemical potential, which clearly uncovers a sign change when the band gap is reversed, which is in good agreement with the experimental observation. The close correlation between the sign of AHE and topological QPT suggests that it can be used as a transport fingerprint for the bulk topology. This is not unexpected given the growing recognition of the topological nature of the intrinsic AHE in recent years (27, 28). The extrinsic AHE, which may be present in realistic materials, is ignored here because it typically dominates in highly metallic materials, whereas the disordered TI films studied here are poorly conductive (20).

The topologically nontrivial FM states with tunable magnetic properties revealed here provide an ideal platform for realizing the exotic magnetoelectric effects proposed by theory. The topology-driven magnetic QPT may also inspire new ideas for topological-magnetic phenomena and spintronic applications in TIs with broken TRS. We cannot completely rule out all other possibilities for the disappearance of FM ordering across the topological QPT. For example, the ARPES results (Fig. 3) show that the properties of itinerant carriers also change with Se content, which may affect an itinerant-driven FM mechanism.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S17
References (29–41)

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Ultrafast Tryptophan-to-Heme Electron Transfer in Myoglobins Revealed by UV 2D Spectroscopy

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Tryptophan is commonly used to study protein structure and dynamics, such as protein folding, as a donor in fluorescence resonant energy transfer (FRET) studies. By using ultra-broadband ultrafast two-dimensional (2D) spectroscopy in the ultraviolet (UV) and transient absorption in the visible range, we have disentangled the excited state decay pathways of the tryptophan amino acid residues in ferric myoglobins (MbCN and metMb). Whereas the more distant tryptophan (Trp^7) relaxes by energy transfer to the heme, Trp^{14} excitation predominantly decays by electron transfer to the heme. The excited Trp^{14} →heme electron transfer occurs in <40 picoseconds with a quantum yield of more than 60%, over an edge-to-edge distance below ~10 angstroms, outcompeting the FRET process. Our results raise the question of whether such electron transfer pathways occur in a larger class of proteins.

The advent of optical-domain multidimensional spectroscopies has opened entirely new perspectives for the study of biochemical dynamics, thanks to their abilities to visualize correlations and interactions between chromophores of the protein (1). This is particularly interesting when it comes to finding the pathways of electron and/or energy transfer in proteins, which are key processes in bioenergetics. Whereas

vibrational multidimensional spectroscopy in the infrared (IR), which detects the couplings between vibrational dipoles, was established in the early 1990s, it was only in mid-2000 that the first study

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of multidimensional electronic spectroscopy appeared using continua in the 800-nm region (2). The authors monitored the transport of energy and the excitonic couplings between seven chromophores of the Fenna-Matthews-Olson photosynthetic light-harvesting protein. The extension of multidimensional spectroscopies to shorter wavelengths is an obvious goal considering the rich variety of biologically relevant chromophores that absorb from the green to the ultraviolet (UV). In partic-

ular, amino acids and nucleic acids absorb below 300 nm. Among the amino acid residues, Trp is found in most proteins and has become a routine probe of protein structure and dynamics using fluorescence resonance energy transfer (FRET). (3–9) Myoglobins contain two Trp residues (Trp¹⁴ and Trp⁷), located in the α helix A (Fig. 1), fluorescence of which is quenched on time scales of 20 to 30 ps and 110 to 140 ps, respectively (10–12). These times are consistent with calculated

quenching rates for a FRET process to the heme. (7, 12) The lack of Trp fluorescence quenching in apomyoglobins was taken as further evidence of FRET to the heme (12).

Trp is also involved in electron transfer processes as the primary donor in DNA repair by photolyase (13), with carbonyl- and sulfur-containing residues (14), and was suggested to reduce Cu(II) in azurin (15). It is also an intermediate in functional multistep hopping processes, for example, in class I ribonucleotide reductase (16), in cytochrome c peroxidase (17), and in azurin tagged with a rhodium carbonyl diimine complex (18).

In general, it is difficult to distinguish electron transfer from FRET if the acceptor does not fluorescence, which is the case with heme proteins. Here, two-dimensional (2D) spectroscopy with broad UV continua [detailed in (19, 20)] is implemented to study the photocycle of ferric CN- and water-ligated horse myoglobins (MbCN and metMb, respectively). This technique allows us to disentangle the overlapping spectral contributions of the various chromophores (Trp⁷, Trp¹⁴, and heme) in the protein. Ferric Mbs are ideal to address the Trp-heme interaction because heme ground-state recovery is complete <10 ps after photoexcitation (21–24). We unravel a Trp¹⁴ $\rightarrow$ heme electron transfer process proceeding in ≤ 40 ps through ~ 8 Å, which efficiently competes with FRET. The Trp-mediated heme reduction is further confirmed by transient absorption spectroscopy in the visible range.

Figure 2 (A to C) shows a set of 2D UV transient absorption spectra of MbCN at different delay times, in which the transient absorption signal is plotted as a function of excitation and detection wavelength (λ). In Fig. 2A, a bleach feature centered at ~ 335 nm appears for $\lambda_{\text{pump}} > 305$ nm, which completely recovers within 10 ps (red dashed trace in Fig. 2E). For $\lambda_{\text{pump}} < 305$ nm, the signal is dominated by excited state absorption (ESA) below $\lambda_{\text{probe}} = 330$ nm, with a dip along the diagonal near 290 nm because of the ground state bleach (GSB) of Trp. The ESA signal persists for over 100 ps. The transition between these two spectral regions reflects the transition from exclusive heme absorption ($\lambda_{\text{pump}} > 305$ nm) to overlapping heme and Trp absorptions ($\lambda_{\text{pump}} < 305$ nm). Thus, the short-lived transient observed for $\lambda_{\text{pump}} > 305$ nm can be ascribed to the heme relaxation, in line with the previously reported <10-ps photocycle (22). The longer-lived features produced by $\lambda_{\text{pump}} < 305$ nm show a significant spectral evolution from tens to hundreds of ps (Fig. 2, B and C). In particular, an ESA signal (Fig. 2C) appears in the 290- to 340-nm region, which differs from the ESA at earlier times (Fig. 2, A and B) because it lacks the absorption below 280 nm, and its maximum is in the region between 305 and 318 nm. It arises exclusively through Trp excitation (Fig. 2E).

To rationalize this complex temporal and spectral evolution, we performed a singular value decomposition and a global analysis (20) of the time-resolved 2D spectra. By modelling the overall kinetics with a multiexponential framework,

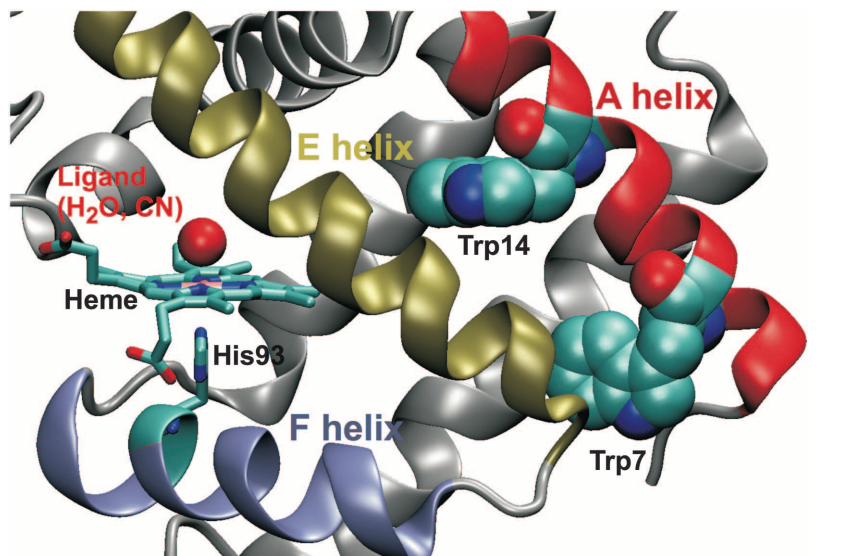


Fig. 1. Molecular structure of horse myoglobin (PDB file 1YMB). The heme; the two Trp residues; the proximal His; and the α helices A, E, and F are highlighted. The oxygen atom of water is shown as a ligand.

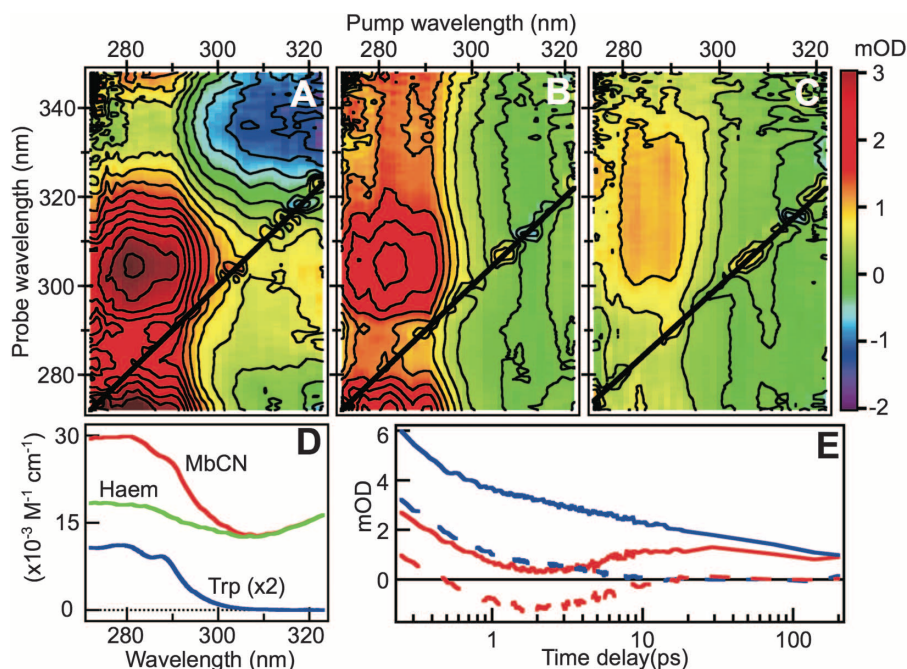


Fig. 2. Two-dimensional pump/probe UV spectra of MbCN at (A) 2.5-, (B) 17-, and (C) 200-ps time delays, plotted with identical color scale [contour line spacing is 0.3 milli-optical density (mOD)]. (D) The steady-state spectrum of MbCN (red) and of tryptophan in water (multiplied by two, blue), and an estimated heme spectrum, obtained by subtracting the absorption spectra of the two tryptophans from the MbCN spectrum (green). (E) Time dependence of the signal at probe wavelengths of 305 (blue) and 335 (red) nm for excitation at 290 (solid traces) and 310 (dashed traces) nm, as extracted from the complete set of 2D spectra.

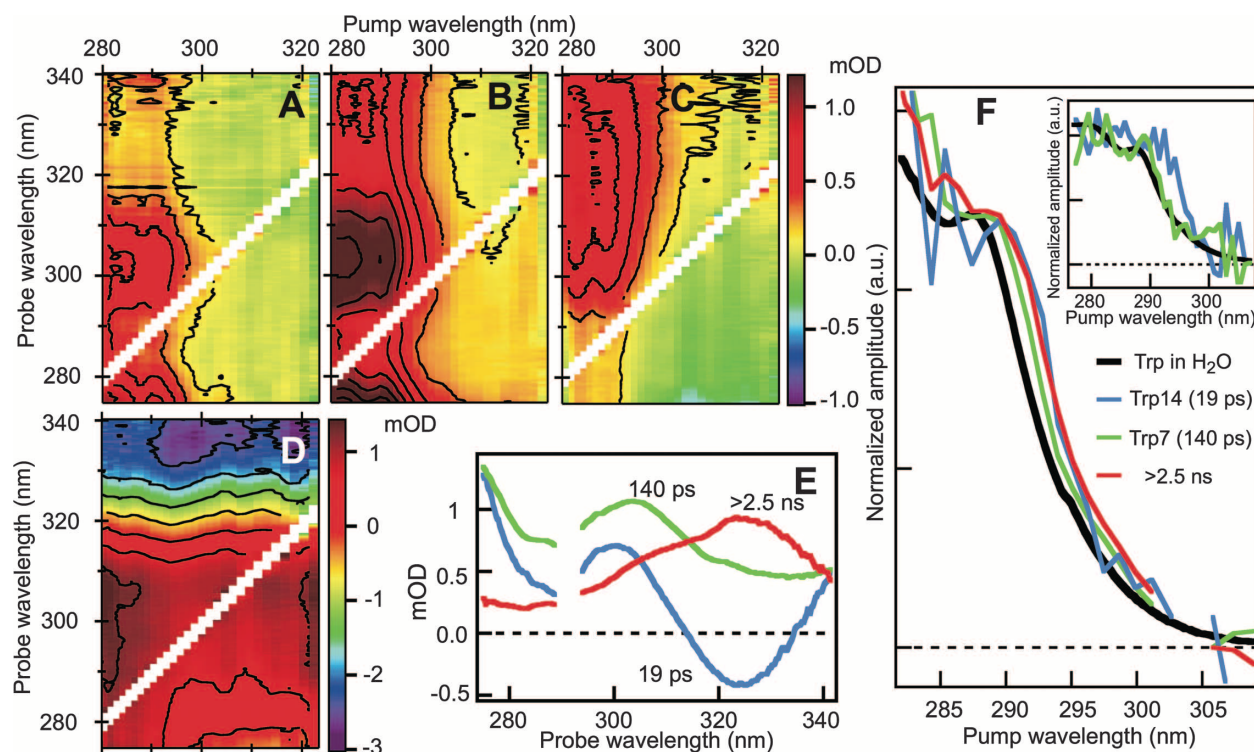


Fig. 3. (A to C) DADAS of MbCN associated with the 19-ps, 140-ps, and ≥ 2.5 -ns components, respectively. Color scale is the same for the three plots; contour line spacing is 0.2 mOD. Points along the diagonal were removed because of scattered light. (D) DADAS associated with the heme relaxation, with a 4.4-ps time constant. The excitation wavelength dependence of the signal reproduces the MbCN heme absorption (see also fig. S2). (E) Cuts of the DADAS in (A) to (C) along the probe axis at $\lambda_{\text{pump}} = 290$ nm. (F) Cuts at $\lambda_{\text{probe}} = 304$ nm along the excitation axis of the (A)

to (C) plots (blue, green, and red lines, respectively). Traces were normalized to the maximum amplitude and are compared to the Trp absorption spectrum in pH = 7 buffer (black). A small shift (0.5 to 1.5 nm) is observed between the Trp⁷ and Trp¹⁴ spectra. The action spectrum of the ≥ 2.5 -ns state agrees well with the absorption spectrum of Trp¹⁴, suggesting that this state is generated by excitation of Trp¹⁴. (Inset) Same but for metMb. The Trp¹⁴ spectrum has a larger red shift (2.5 to 3 nm) compared with Trp⁷ than in MbCN [see (20) for details]. a.u., arbitrary units.

the characteristic time constants and respective amplitudes were obtained for each pair of (λ_{pump} , λ_{probe}) wavelengths [Fig. 3 and figs. S1 (MbCN) and S3 (metMb)]. The plots in these figures constitute the decay-associated dispersed action spectra (DADAS), which are the 2D assemblies of decay-associated spectra (DAS) [see (20) for more details]. A cut of these spectra along the probe axis provides the conventional (1D) DAS, that is, the spectra associated with the respective time constants for a fixed excitation wavelength. A cut along the excitation axis provides the action spectrum of the observed kinetics.

Six time constants are necessary to describe the observed transients. For MbCN, the three extracted fast components, <300 fs, 1.1 ± 0.1 ps, and 4.4 ± 0.2 ps (error bars represent standard deviations, table S1 and fig. S1), are in good agreement with previous ultrafast visible and IR studies (22, 24, 25). These components are detected at all excitation wavelengths, and their action spectra (Fig. 3D and figs. S1 and S2) agree with the MbCN heme absorption; that is, they reflect the heme relaxation.

Three additional time constants (19 ± 1 ps, 140 ± 10 ps, and ≥ 2.5 ns; table S1 and Fig. 3, A to C) are necessary to describe the system kinetics for excitation below ~ 305 nm. The 19- and 140-ps components agree with the known Trp¹⁴ and Trp⁷

fluorescence decays, respectively (7, 11, 12). The transient spectra along the λ_{probe} axis are dominated by the Trp ESA, with two strong absorption features at ~ 303 nm and below 275 nm, separated by a minimum because of the Trp GSB. The same pattern of results and time scales is found for metMb (table S1 and fig. S3).

The ≥ 2.5 -ns (>0.7 -ns in metMb) component, in contrast, was not reported before. Although this state is only produced by excitation below ~ 305 nm, its probe spectrum (Figs. 2C and 3, C and D) is significantly different from the 19- and 140-ps DAS. None of the Trp ESA bands are present, whereas an absorption band appears at 325 nm with a shoulder at 307 nm (Fig. 3E). This strongly suggests that the state responsible for this transient cannot be associated with an excited Trp. The 19-ps DAS (Fig. 3E) contains, in addition to the typical features of the Trp decay (see trace of the 140-ps DAS), a component that matches the mirror image of the ≥ 2.5 -ns DAS, indicating a population growth of the long-lived state with a time scale of 19 ps, suggesting that it is generated by the decay of Trp¹⁴. The data analysis for metMb yields similar temporal and spectral behaviors (fig. S3 and table S1).

Figure 3F shows typical cuts of the >10 -ps DADAS. For the decay of the two Trp molecules and the 2.5-ns component, the traces are in

agreement with the spectral shape of the Trp steady-state absorption in a pH = 7 buffer, but they all show a small red shift with respect to it. The Trp¹⁴ absorption and the action spectrum of the ≥ 2.5 -ns component coincide and show a small red shift (~ 1 nm) with respect to the Trp⁷ absorption. A similar effect is observed also in metMb (Fig. 3F, inset), but the shift of the Trp¹⁴ absorption is larger (2.5 to 3 nm). This is briefly discussed in the supplementary materials.

The good agreement of the action spectrum of the long-lived state with the Trp¹⁴ absorption confirms that this state is populated by the decay of excited Trp¹⁴. To identify the nature of this long-lived photoproduct, we carried out (1D) visible transient white-light probe measurements upon 290-nm excitation. Figure 4A shows the time-wavelength plot for MbCN (fig. S5 for metMb). In the Q-band region (500 to 600 nm), a double-peaked structure grows on a 20-ps time scale and lasts for over 700 ps (the limit of our measurement). In the Soret-band region (380 to 450 nm), a similar rise is observed for negative and positive transient bands, centered at 419 and 438 nm, respectively. The spectral shape of this long-lived state (Fig. 4B) agrees well with the difference between ferrous and ferric MbCN spectra, indicating the formation of a ferrous-MbCN (metMb) photoproduct (26). The reduction quantum yield

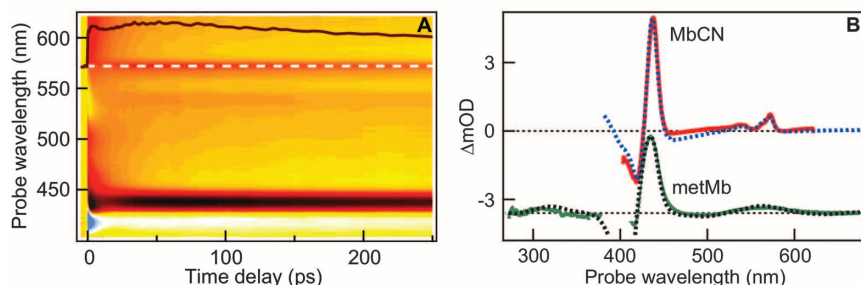


Fig. 4. (A) Visible transient absorption after 290-nm excitation of MbCN. Positive signals are plotted in red; negative features appear blue. Signal intensity is shown by using an asinh scale, which enhances the weak features between 500 and 600 nm, where two narrow bands rise within the first 50 ps. The solid line shows the kinetic trace at $\lambda_{\text{probe}} = 572$ nm (dotted line). A similar rise of the signal is present in the region of the Soret band (400 to 450 nm). (B) Comparison between the spectral shape of the long-lived signal observed in MbCN (red) and the difference between ferrous (Fe^{2+}) and ferric MbCN spectra (blue dotted line) (26). Because of the short lifetime (~ 2 min at -5°C) of Fe^{2+} -MbCN, static measurements below 350 nm, where the reductant ($\text{Na}_2\text{S}_2\text{O}_4$) absorbs, are not available. The spectral shape of the long-lived transient in metMb (green markers) shows good agreement with the difference between deoxy-Mb and the ground-state absorption (black dotted line). Traces are vertically shifted by -0.36 OD for clarity. Points between 375 and 415 nm were removed because of distortions from the high optical density of the sample. Displayed signals are normalized to the same number of excited molecules.

(QY_T) upon 290-nm excitation is estimated from the fraction of excited molecules and the number of photoreduced molecules, which is in turn obtained from the amplitude of the DAS associated with the long-lived state compared with the difference between the reduced and nonreduced MbCN (metMb) absorption spectra (see supplementary materials). We find a quantum yield of 11 to 12% (corresponding to a reduction cross section $\sigma_{\text{red}} = \sigma_{\text{abs}} \text{QY}_T \approx 2 \times 10^{-17} \text{ cm}^2$) in both MbCN and metMb. Under static conditions $\sigma_{\text{red}} \approx 5 \times 10^{-19} \text{ cm}^2$ was measured for metMb under 290-nm irradiation (27), which implies that most of the hemes photoreduced in our experiment re-oxidize on time scales beyond our measurement window. In fact, for both samples a decay of the reduced state occurs on a 100- to 200-ps time scale, which accounts for only 30 to 40% of the photo-reduced hemes (Fig. 4 and figs. S4 and S5). This may indicate a partial, fast back electron transfer. The Trp cation radical is likely to be produced in an excited state from the Trp-to-heme electron transfer [similar to (28)]. A branching between deprotonation and intersystem crossing to the ground state in its relaxation may explain the partial electron hole recombination: TrpH^+ in the ground state would allow for a fast back electron transfer, whereas deprotonated Trp would prohibit it. Such an explanation is reasonable but needs further confirmation.

The above data unambiguously support reduction of the heme by Trp^{14} , overwhelming the FRET process. No detectable electron transfer from Trp^7 is found. At 290 nm, Trp^{14} excitation accounts for $\sim 18\%$ of the total number of excited molecules (29). Thus, the quantum yield (QY) of the $\text{Trp}^{14} \rightarrow \text{heme}$ electron transfer is $\geq 60\%$ in MbCN and $>50\%$ in metMb. The time constants for electron transfer obtained from the QY and the observed decay times of excited Trp^{14} in MbCN (metMb) are <34 ps (<40 ps).

Long-range (>5 Å) electron transfer in proteins occurs either by tunnelling through covalent bonds

forming the secondary structure (21, 30) or by hopping across specific intermediates (13, 16, 17, 31). The distance dependence of the tunnelling probability through the protein medium follows an exponential behavior $\exp(-\beta r)$ (32), where β can be empirically determined, and differs for different protein secondary structures (33). Pathway- (34) and density-packing (35, 36) models account in more detail for the molecular protein structure. The edge-to-edge distance between Trp^{14} and the porphyrin ring is ~ 8 Å; the distance to the Fe atom is ~ 12 Å. Our observed rate is consistent with Marcus theory (21, 37) assuming a well-optimized process (Gibbs free energy of the redox reaction equals reorganization energy) and $\beta \sim 1.1$ to 1.3 Å^{-1} , which is in good agreement with density-packing predictions (35). The pathway analysis (34, 38) allowed determination of a large number of electron transfer paths that are of relevance here. The electron transfer is mediated by the residues of the α -helix E located between the heme and the indole ring of Trp^{14} and in (or close to) van der Waals contact with the latter (Fig. 1 and fig. S7). The estimated time of >40 ns for electron transfer through the ~ 13.5 Å Trp^7 -heme edge-to-edge distance, compared with the 140-ps FRET time, explains the dominance of the latter. Although it is not clear whether electron transfer also occurs in ferrous myoglobins, the invariance of the Trp^{14} lifetimes with redox state and nature of ligand (11) strongly suggests this to be the case.

The present studies, along with the examples of Trp-mediated electron transfer in other proteins (13–18), raise questions about its widespread use in FRET studies of protein dynamics, especially when the acceptor is not fluorescent.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S6

Table S1

References (39–45)

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Tuning Selectivity in Propylene Epoxidation by Plasmon Mediated Photo-Switching of Cu Oxidation State

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Oxidation of functioning copper has restricted its applicability as a catalyst for commercially important epoxidation of propylene to form propylene oxide. Here, we report that steady-state selectivity in propylene epoxidation on copper (Cu) nanoparticles increases sharply when the catalyst is illuminated with visible light. The selectivity increase is accompanied by light-induced reduction of the surface Cu atoms, which is brought about by photoexcitation of the localized surface plasmon resonance (LSPR) of Cu. We discuss multiple mechanisms by which Cu LSPR weakens the Cu-O bonds, reducing Cu₂O.

Heterogeneous catalysts evolve under operating conditions to a steady chemical state that can manifest distinct reactivity from the initial, as-prepared material. For instance, ultrahigh-vacuum (UHV) experiments on well-defined copper (Cu) single-crystal surfaces have shown that metallic Cu exhibits high selectivity to epoxide products in the epoxidation of multiple olefins (1–4). However, under realistic conditions for commercially critical epoxidation of propylene to form propylene oxide ($\text{C}_3\text{H}_6 + 1/2\text{O}_2 \rightarrow \text{C}_3\text{H}_6\text{O}$), characterized by equimolar amounts of propylene and oxygen and temperature of ~400 to 500 K, Cu is oxidized (4–6). On the oxidized Cu surface, the selectivity to the desired propylene oxide (PO) product is very low.

We demonstrate an approach to manipulate the oxidation state of Cu catalysts under operating propylene epoxidation conditions and thereby tune the selectivity of the catalyst to favor PO production. The approach takes advantage of the strong interactions of Cu nanoparticles with visible light manifested in the photoexcitation of the localized surface plasmon resonance (LSPR) (7–9). We find that heterogeneous catalysts containing Cu nanoparticles exhibit a sharp increase in the steady-state selectivity to propylene epoxide from ~20 to ~50% when illuminated with visible light. This increase in the selectivity is accompanied by a light-induced change in the oxidation state of the surface Cu atoms from Cu-oxide (light off) to Cu metal (light on). We find that at a critical light intensity threshold, the change in the product selectivity and Cu oxidation state are observed within less than 2 min, which was the detection limit of our experimental setup.

Catalyst preparation and the experimental setup are described in detail in the supplementary materials. In short, catalysts (12 mg) contained 2 weight percent of Cu nanoparticles, with an average size of 41 ± 9 nm (fig. S1) supported on an inert SiO₂ support and tightly packed into a packed bed reactor. The reactant stream contained

equimolar amounts of propylene and oxygen at a total flow rate of 100 cm³/min and atmospheric pressure. A broadband visible light source with maximum spectral intensity at ~580 nm (fig. S2) and tunable power was used in all experiments. Reaction rates and product selectivity were measured in the differential reactor setup in the regime limited by the reaction kinetics under steady-state reaction conditions.

Steady-state PO selectivity and rate of propylene consumption measured at 473 K as a function of light intensity is shown in Fig. 1A. The data show that the PO selectivity—measured as the rate of generation of PO divided by the rate of generation of all products (equivalent to the rate of consumption of propylene) per unit mass of the Cu catalyst—is ~20% [with the balance consisting of CO₂ and acrolein (fig. S3)] for visible light intensity below ~550 mW/cm² (~4 to 5 suns). The selectivity to PO rapidly increases to ~50% as the light intensity crosses the threshold of ~550 mW/cm². This is followed by a decrease in the PO selectivity, with a further increase of

light intensity, reaching ~35% at ~800 mW/cm². The data also show that the reaction rate, measured as the rate of propylene consumption per unit mass of the Cu catalyst, increases for intensities up to 550 mW/cm² followed by a sharp decrease at ~550 mW/cm² and then an increase for intensity above 550 mW/cm². We observed the same threshold behavior for the range of operating temperature between 473 and 553 K.

To obtain an unbiased measure of product selectivity, it is important to measure it at constant rates of propylene consumption. In a steady-state flow reactor, the product selectivity at constant rate is directly proportional to the product yield. At constant temperature and propylene consumption rate, the catalyst illuminated with intensity higher than ~550 mW/cm² has higher PO selectivity than that of the catalyst illuminated with intensity below ~550 mW/cm² (Fig. 1A). For example, for the propylene consumption rate of ~8 μmol/g/s, measured at intensities of 150 and 550 mW/cm², the ~50% PO selectivity for the larger (550 mW/cm²) intensity is much greater than the only 18% selectivity for the 150 mW/cm² intensity. Similarly, for the propylene consumption rates of 25 and 45 μmol/g/s—achieved at (~400 and 700 mW/cm²) and (450 and 800 mW/cm²), respectively—the PO selectivity is larger for the light intensity above the 550 mW/cm² threshold. This is actually even the case for all reaction rates at constant temperature; the data in Fig. 1A show that the highest measured PO selectivity for the surface illuminated with the intensity below 550 mW/cm² is lower than the lowest PO selectivity obtained for intensities above 550 mW/cm². In Fig. 1B (as well as figs. S4 and S5), we compared the PO selectivity for the thermal process (no light illumination) with the selectivity on identical catalysts illuminated with the visible light at 550 mW/cm² at constant reaction rates. In these experiments, the rate was manipulated by

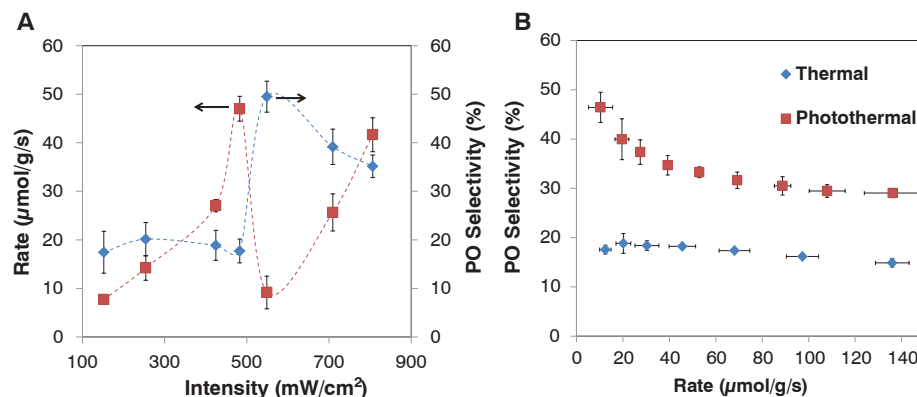


Fig. 1. (A) Rate of propylene consumption (left axis, red squares) and the selectivity to PO (right axis, blue diamonds) under photothermal conditions (light on) at 473 K as a function of light intensity. The rate and selectivity data shown in (A) represent the average values of the data obtained from five independent experiments, and the error bars represent SD. (B) Selectivity to PO for thermal (light off) and photothermal (light on) processes as a function of the reaction rate. The light intensity used for the photothermal studies was 550 mW/cm². The reaction rate and selectivity data shown in (B) represent the average values of the data obtained from three independent experiments, and the error bars represent SD.

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adjusting the temperature rather than by adjusting the light intensity. The data show that for all measured rates, the steady-state PO selectivity and therefore the PO yield is higher for the illuminated catalyst (light intensity ≥ 550 mW/cm²).

The data in Fig. 1 and figs. S3, S4, and S5 show that the steady-state catalytic process undergoes a dramatic change, manifested as a rapid increase in the intrinsic PO selectivity, as the light intensity threshold of ~ 550 mW/cm² is crossed. The data also show that at constant temperature, for the light intensity below ~ 550 mW/cm² the rate of propylene consumption increases with light intensity, followed by a sharp decrease at the threshold of 550 mW/cm² and a subsequent increase above the threshold intensity. The light intensity threshold will be affected by multiple factors, including the operating partial pressures of reactants, temperature, and the packing of metallic nanoparticles.

As stated above, UHV studies on model systems have shown that the oxidation state of surface Cu atoms affects the selectivity of Cu-based catalysts in epoxidation reactions, with metallic Cu yielding higher epoxide selectivity than that of the oxidized Cu surface (1–4). To test whether the light-induced reduction of Cu-oxide to Cu metal might be responsible for the observed increase in the PO selectivity, we characterized the Cu catalysts using in situ ultraviolet-visible (UV-vis) extinction spectroscopy. In Fig. 2A, we show the extinction spectrum of the catalyst, containing nanoparticles of Cu on SiO₂, reduced in 20% H₂ (balance helium) at a temperature of 523 K and atmospheric pressure. It is well established that the extinction peak at ~ 565 nm is a signature of metallic Cu nanoparticles (fig. S6) (10, 11). The extinction is due to the excitation of

the LSPR in metallic Cu nanoparticles. The data also show that as the metallic Cu nanoparticles are exposed to 20% oxygen in helium at 473 K and atmospheric pressure, the LSPR extinction peak is broadened and red shifted, whereas the extinction intensity decreases. These changes in the extinction spectrum upon exposure of Cu nanoparticles to oxygen have been characterized, and it has been shown that they are the result of the formation of a shell of Cu₂O oxide on the metallic Cu core (10, 11). Because of the high sensitivity of Cu LSPR to the local chemical environment, changes in the oxidation state of even a small fraction of Cu surface atoms are accompanied by substantial changes in the extinction spectrum (12–14).

The UV-vis extinction spectrum of the functioning Cu catalyst at steady-state conditions is also shown in Fig. 2A. These catalysts were first prereduced in H₂ and subsequently exposed to operating propylene epoxidation conditions. The data show that as the reactants—equimolar amounts of propylene and oxygen at atmospheric pressure—are introduced at 473 K, the UV-vis extinction spectrum changes from that associated with the metallic Cu to that of the oxidized Cu shell covering the metallic core. Subsequent illumination of the functioning Cu catalyst with UV-vis light of 550 mW/cm² intensity results in a dramatic transformation of the catalyst, manifested as the reduction of the Cu-oxide shell. The oxidation (light off) and reduction (light above 550 mW/cm²) of the functioning catalyst take place within 2 min, which is the time required to measure the UV-vis extinction spectrum and well below the time it takes to start making steady-state measurements. As shown in Fig. 1, this light-induced change in the chemical state of surface Cu atoms at

550 mW/cm² is accompanied by a dramatic increase in the PO selectivity and a drop in the reaction rate. The conclusion of the in situ UV-vis extinction measurements were further supported by an ex situ analysis of x-ray diffraction (XRD) patterns of the selective (light at 550 mW/cm² on) and unselective (light off) catalysts. The unselective catalysts exhibit diffraction features that are consistent with the presence of the Cu₂O shells on Cu, whereas the selective catalysts do not exhibit the oxide diffraction features (Fig. 2B) (10).

To further explore the origin of the observed light-induced change in the oxidation state of the Cu catalysts and accompanying changes in the reaction rates and selectivity, we studied the behavior of illuminated, functioning Cu catalysts as a function of the wavelength of incident light. Multiple short- and long-pass optical filters were used to control the wavelength of light reaching the catalysts from the broadband visible light source. The total intensity of light reaching the catalyst was ~ 550 mW/cm² in all experiments, and the operating temperature was 473 K. In the presence of 500-nm long-pass filters (mainly light with wavelengths longer than 500 nm reaching the catalysts), the above-discussed surface transformation from oxidized Cu to metallic Cu takes place (Fig. 3A). On the other hand, as shown in Fig. 3B, when a 500-nm short-pass filter is applied (mainly photons below 500 nm reaching the catalyst) the catalyst is oxidized under the reaction conditions and unperturbed by light. This result suggests a critical role for the Cu LSPR in inducing the chemical transformation of Cu and driving the observed light-induced increase in the PO selectivity because photons with the wavelength below 500 nm do not excite the Cu LSPR.

We propose that the photoexcitation of Cu LSPR, associated with the metallic core of catalytic nanoparticles, serves to reduce the Cu₂O shell at the threshold light intensity of 550 mW/cm² and maintain the Cu surface in the metallic state under operating conditions. At this threshold intensity, the material undergoes a dramatic phase change from an oxide to a metal—the material becomes different on the atomic level. Below and above the threshold intensity, the chemical process is taking place on two atomically different surfaces: oxide and metal, respectively. It is this light-induced change in the atomic structure of the surface that causes the dramatic noncontinuous change in the rate of propylene consumption and the intrinsic product selectivity. This noncontinuous change in product selectivity and the rate of propylene consumption observed at the threshold intensity is fundamentally different than the observed variation in the rate and selectivity for intensities below and above the threshold. For light intensities below the threshold, an increase in the intensity results in a continuous increase in the rate of propylene consumption. As is the case in almost any selective partial oxidation reaction, higher conversion of reactant yields higher

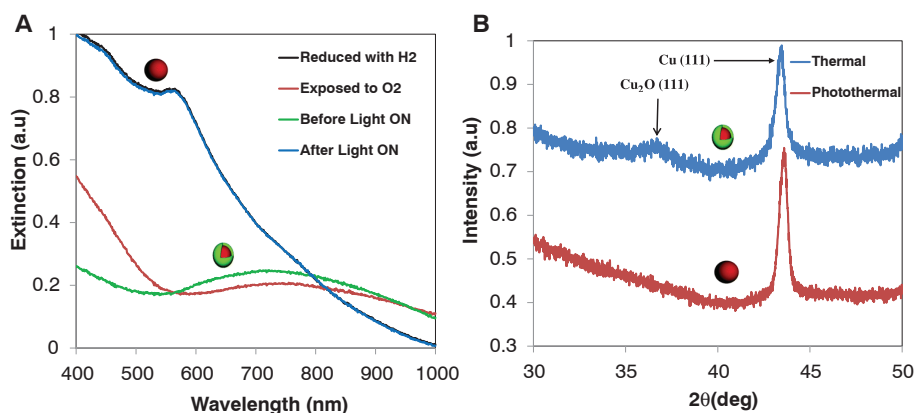


Fig. 2. (A) In situ diffuse reflectance UV-vis extinction spectra of the catalyst (2% Cu/SiO₂) reduced with hydrogen at 523 K, exposed to oxygen at 473 K, under propylene epoxidation conditions without light (before light on) at 473 K, and under propylene epoxidation conditions with light on (after light on) at 473 K. The light intensity used for the photothermal studies (after light on) was 550 mW/cm². The UV-vis extinction spectra of the catalyst reduced with hydrogen and exposed to propylene oxidation under photothermal conditions (after light on) almost completely matched each other. To distinguish these two spectra, the extinction spectrum of the catalyst reduced with hydrogen was scaled by 1.01. a.u., arbitrary units. (B) X-ray diffraction spectra for the catalyst used in thermal (light off) and photothermal (light on) studies. The light intensity used for the photothermal studies was 550 mW/cm². The arrows indicate the peak positions of Cu(111) and Cu₂O(111) signals.

selectivity to the deep oxidation product CO_2 . In this particular case, as the rate of propylene consumption increases, the partial pressures of acrolein and PO in the reactor increase, causing the rate of sequential combustion of these species to increase and yielding higher selectivity to CO_2 . The data in Fig. 1A and fig. S3 show that on the Cu oxide surface (intensity below the threshold), the main channel contributing to the increased selectivity to CO_2 at higher rates of propylene consumption involves the sequential oxidation of acrolein—the increase in the CO_2 selectivity is accompanied by a concurrent drop in the acrolein selectivity.

The data in fig. S5 show that a similar effect on the rate of propylene consumption and product selectivity is achieved by increasing the temperature of the Cu oxide surface (light off). This suggests that on the oxide surface, the change in selectivity is only the consequence of the enhanced rate of propylene consumption, and it makes no difference whether the change in the rate is induced by increasing the operating temperature or increasing the intensity of light. Similarly, for light intensities above the threshold an increase in the light intensity also results in an increase in the rate of propylene consumption, therefore increasing the partial pressure of acrolein and PO in the reactor. Again, an increase in the rate of sequential combustion of these species results in higher selectivity to CO_2 as the light intensity is increased. The data in Fig. 1A show that on Cu metal (intensity above the threshold), the main channel contributing to the increased selectivity to CO_2 involves the sequential oxidation of PO. The data in fig. S4 show that a similar effect on the reaction rate and product selectivity is achieved by increasing the temperature of the Cu metal surface.

We also must comment on the mechanism involved in the reduction of the Cu_2O oxide shell. Cu_2O is a semiconductor with a band gap of ~ 2.0 eV (15). There are two mechanisms by which Cu LSPR could induce the reduction of Cu_2O . First, radiative energy transfer from the plasmon Cu states to the oxide shell enhances the rate of electron excitation from the valence (bonding orbitals) to conduction band (antibonding orbitals) of the Cu-oxide shell, weakening the Cu-O chemical bonds in the oxide. This plasmonic energy transfer from an excited plasmonic metal to a nearby semiconductor is well documented (7, 16–20). It takes place mainly via dipole-dipole interactions of intense LSPR-mediated electromagnetic fields around the Cu metallic core with the oxide shell. In this mechanism, the metallic Cu core essentially traps light, locally amplifying its effect on the semiconductor (7, 16–20). Second, direct LSPR-mediated transient injection of electrons from the metallic Cu core to the antibonding Cu-O orbitals of the oxide shell would also lead to a more facile reduction of Cu-oxide shell. The process has been shown to be functional in many systems in which energetically accessible electronic states (orbitals) interact

with metal LSPR. The process of electron injection takes place either directly via chemical interface damping (CID) (7–9, 21–25) of the metal plasmon states because of their interaction with the antibonding Cu-oxide states, or indirectly through the decay of plasmons into hot electrons (7–9, 25–29) via Landau damping and subsequent transfer of the energetic electrons to Cu_2O . The observed broadening of LSPR resonance upon the formation of the Cu-oxide shell

(Fig. 3) is a clear indication that the imaginary component of the material dielectric function increases, which is direct evidence for the faster rates of LSPR relaxation, most likely owing to the direct injection of electrons into the oxide semiconductor (21–24). Alternatively, the Cu LSPR might conceivably lead to heating of the Cu particles and their thermal reduction (25). This mechanism is not likely because as shown in Fig. 4, if we thermally heat the catalyst (light

Fig. 3. Spectra of the light source with (A) 500-nm long-pass filter and (B) 500-nm short-pass filter used in the photothermal studies, superimposed with diffuse reflectance UV-vis extinction spectra of catalyst (2% Cu/SiO₂) measured in situ at 473 K under thermal (before light on) and photothermal (after light on) conditions. The light intensity used for the photothermal studies was 550 mW/cm².

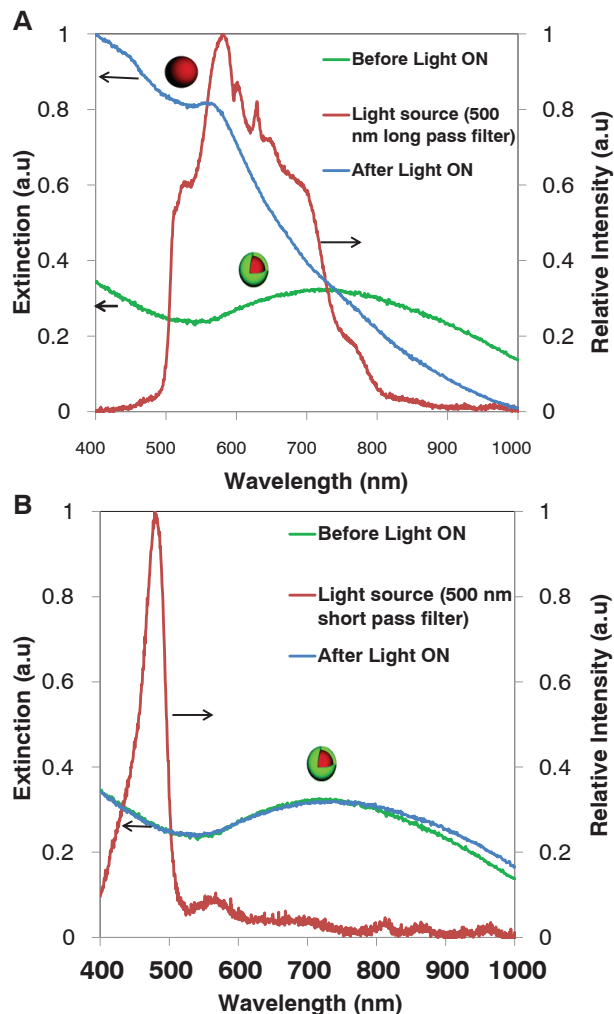
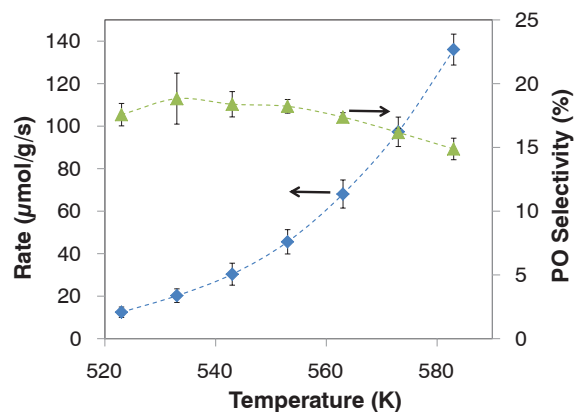


Fig. 4. Rate of propylene consumption (left axis, blue diamonds) and selectivity to PO (right axis, green triangles) under thermal conditions (light off) as a function of temperature. The rate and selectivity data shown in the figure represent the average values of the data obtained from three independent experiments, and the error bars represent SD.



off) the reaction rate steadily increases, and the PO selectivity steadily decreases, as a function of temperature. This behavior is fundamentally different than the behavior observed in response to light (Fig. 1, A and B), in which we see sharp drops in the reaction rate and a sharp increase in the selectivity at ~ 550 mW/cm².

The studies discussed advance a number of critical concepts. Although we focused on Cu nanostructures, the discussed mechanisms are universal, and similar principles could be used in the design of various metal nanomaterials with photo-switchable oxidation states. For example, core-shell nanoparticles containing a plasmonic core (such as Au, Ag, or Cu) and a shell of another metal could lend themselves to similar LSPR-mediated photo-induced switching of the oxidation states of surface atoms. Controlling the oxidation state of functioning catalysts is critical for the control of reactant conversion rates and product selectivity. Second, direct epoxidation of propylene without expensive sacrificial agents is one of the most important processes for which no viable heterogeneous catalyst exists. Although the findings reported here may pave the way toward the discovery of viable heterogeneous propylene epoxidation catalysts, hurdles related to efficient and scalable harvesting of light (including abundant solar light) represent considerable challenges.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S6
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Photoredox Activation for the Direct β -Arylation of Ketones and Aldehydes

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The direct β -activation of saturated aldehydes and ketones has long been an elusive transformation. We found that photoredox catalysis in combination with organocatalysis can lead to the transient generation of 5π -electron β -enaminyl radicals from ketones and aldehydes that rapidly couple with cyano-substituted aryl rings at the carbonyl β -position. This mode of activation is suitable for a broad range of carbonyl β -functionalization reactions and is amenable to enantioselective catalysis.

Within the field of organic chemistry, the carbonyl moiety is central to many broadly used synthetic modifications and fragment coupling steps, including Grignard reactions, Wittig olefinations, and reductive aminations. The carbonyl system is also a preeminent activation handle for proximal bond constructions such as α -C=O oxidations, alkylations, arylations, and halogenations (1, 2). However, transformations that allow the direct β -functionalization of saturated ketones and aldehydes remain effectively unknown (3, 4). Herein, we describe a catalysis activation mode that arises from the combination of photoredox and amine catalysis to enable

the direct arylation of cyclic and acyclic carbonyls at the β -methylene position (Fig. 1).

Historically, β -carbonyl activation has been limited to the addition of soft nucleophiles to α,β -unsaturated carbonyls. Although the study of such reactivity has delivered many useful transformations, this chemistry requires substrates that are not as widely available or require an additional preoxidation step from a saturated precursor (5–9). Previous studies in transition metal catalysis have shown that direct β -functionalization of esters or amides is possible through the use of palladium catalysis, albeit with only a few examples known to date (10–13). A direct aldehyde or ketone β -coupling mechanism would obviate the need for preactivated substrates.

Over the past 4 years, visible light-mediated photoredox catalysis has become a rapidly blossoming research area within the organic and organometallic communities (14–17). Through a series of photoinduced electron transfer (PET) events, this general strategy allows for the development of bond constructions that are often elusive or currently impossible via classical two-electron pathways. In addition, photoredox catalysis provides an alternative means of generating reactive radical intermediates without operational complexity and toxic precursors (often found with high-energy photochemistry and/or reagent-based radical production).

Recently, using the concept of “accelerated serendipity,” our laboratory discovered a unique bond construction that enables the direct arylation of α -methylene amines via visible light photoredox catalysis (18). On the basis of mechanistic insights gained from this arylation protocol, we hypothesized that photoredox and amine catalysis might be used in conjunction to enable the direct β -functionalization of carbonyls. A prospective mechanism for this dual-catalysis β -aldehyde or ketone arylation is shown in Fig. 2. It is well known that tris-cyclometalated Ir(III) complexes, such as tris(2-phenylpyridinato- C^2,N)iridium(III) [Ir(ppy)₃] (1), have a strong absorption cross section in the visible range, allowing them to accept a photon from a variety of light sources to populate the ^{*}Ir(ppy)₃ (3) metal-to-ligand charge transfer excited state (19). Given that ^{*}Ir(ppy)₃ (3) is a strong reductant [oxidation potential ($E_{1/2}^{ox}$) = –1.73 V versus saturated calomel electrode (SCE) in CH₃CN] (19, 20), we proposed that this high-energy

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intermediate would undergo single-electron transfer (SET) with 1,4-dicyanobenzene (**4**) [reduction potential $E_{1/2}^{\text{red}} = -1.61$ V versus SCE in CH_3CN] (**21**) to afford the arene radical anion **5** and oxidized photocatalyst $\text{Ir}^{\text{IV}}(\text{ppy})_3$ (**6**). Concurrent with this photoredox step, we hypothesized that amine catalyst **2** should condense with aldehyde substrate **7** to form the electron-rich enamine **8**. At this juncture we hoped that the photoredox and organocatalytic cycles would merge, as the electron-deficient $\text{Ir}^{\text{IV}}(\text{ppy})_3$ (**6**) intermediate ($E_{1/2}^{\text{red}} = +0.77$ V versus SCE in CH_3CN) (**19**, **20**) should readily accept an electron via SET from the electron-rich enamine **8** to generate the corresponding radical cation **9** (and thereby complete the photoredox cycle) (**22**). As a key mechanistic consideration, we hypothesized that formation of the enaminy radical cation **9** would sufficiently weaken the allylic C–H bonds (at the original carbonyl β -position) so as to allow deprotonation and formation of the β -enamine radical **10**, which represents the critical $5\pi e^-$ activation mode. We assumed that intermolecular coupling of radical anion **5** and β -radical **10** would then forge a new $\text{sp}^3\text{-sp}^3$ carbon-carbon bond and that the resulting cyclohexadienyl anion **11** would undergo rapid β,δ -elimination of cyanide to regain aromaticity. Hydrolysis of the resulting enamine would then complete the organocatalytic cycle while delivering the desired β -aryl aldehyde product **12**.

This dual-catalysis design plan has several key features. First, a stabilized radical, such as radical anion **5**, would be sufficiently electron-rich to avoid direct reaction with enamine **8**, thus avoiding bond formation at the α -carbonyl position. Similarly, electron-deficient arenes, such as 1,4-dicyanobenzene, are capable of forming radical anion intermediates that have relatively long lifetimes, thereby increasing the propensity for selective radical-radical bond formation with the β -enaminy radical **10** (**23**). Moreover, such radical anion species do not typically engage in homocoupling—a step that would lead to a net loss in reaction efficiency. We also recognized from an early stage that the requisite amine catalyst must be sufficiently electron-rich to chemically enable three critical steps: (i) enamine formation, (ii) enamine oxidation, and (iii) nucleophilic radical coupling of the key $5\pi e^-$ activated intermediate **10**. With respect to the key deprotonation step (**9** to **10**), we recognized that an alternative mechanism might be encountered wherein the nitrogen-centered radical cation **9** could undergo proton loss at the α -methylene position on the catalyst framework to generate an α -amino radical (not shown). However, initial density functional theory calculations to investigate this chemoselectivity issue suggested that the proposed allylic C–H bond functionalization has a significantly lower energy of activation than the corresponding α -aminium radical deprotonation step (**24–26**).

With these considerations in mind, we first examined this β -carbonyl arylation reaction with

octanal, 1,4-dicyanobenzene, and a variety of amine catalysts, photocatalysts, and weak light sources. To our delight, we found that the use of $\text{Ir}(\text{ppy})_3$ (**1**) and *N*-isopropylbenzylamine (*i*-PrBnNH) (**13**) in the presence of a 26-W fluorescent light bulb while using 1,4-diazobicyclo[2.2.2]nonane (DABCO) as a base provided the respective β -aryl aldehyde adduct in 86% yield, without formation of any α -amine arylation adducts. Control experiments established the importance of both

the organocatalyst and the photocatalyst, as no desired reaction was observed in the absence of light, $\text{Ir}(\text{ppy})_3$, or *i*-PrBnNH (**26**). The fact that $\text{Ir}(\text{ppy})_3$ was found to be the optimal photocatalyst is readily appreciated given its matched redox potentials for both the reduction of 1,4-dicyanobenzene and oxidation of the amine catalyst-derived enamine.

Having identified the optimal conditions for this β -arylation reaction, we next examined the

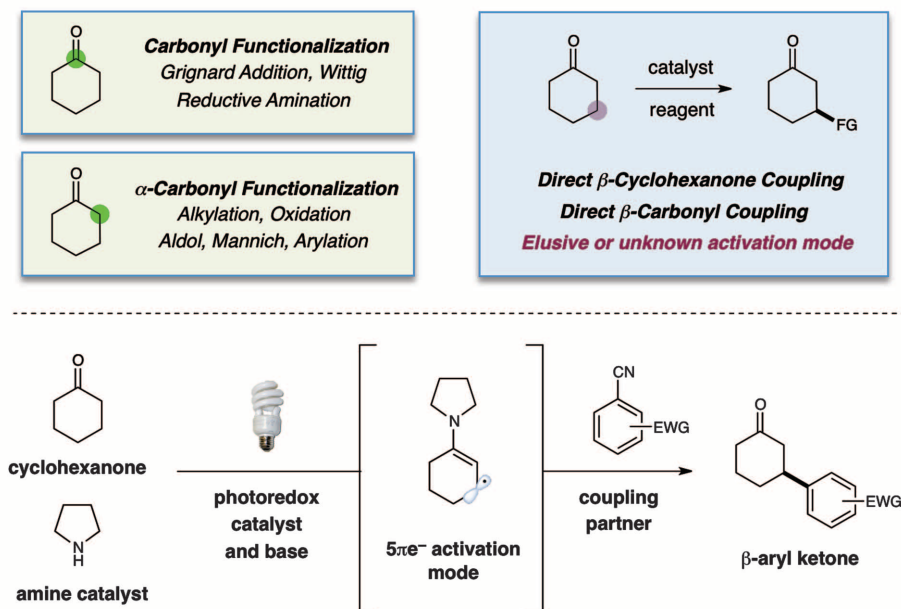


Fig. 1. Proposed activation mode for direct β -arylation of carbonyls—an elusive transformation.

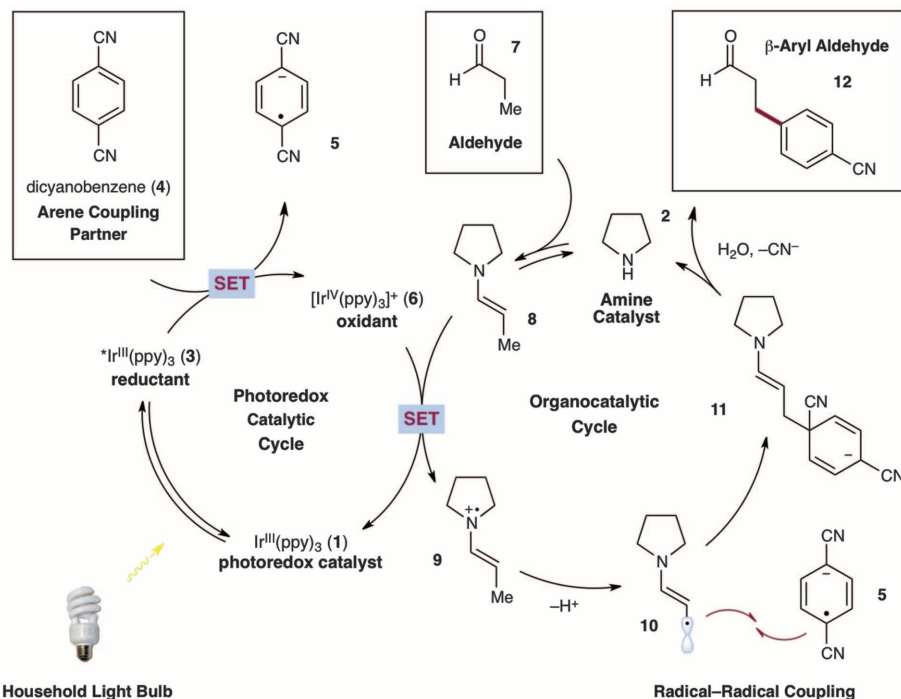


Fig. 2. Photoredox C–H β -arylation: Proposed mechanistic pathway (Me, methyl).

scope of the aldehydic coupling partner. As shown in Fig. 3, a broad array of alkanals can serve as competent substrates. For example, functional groups as diverse as ethers, alkenes, alkynes, arenes, and carbamates are readily tolerated (Fig. 3, entries 17 to 22; 63 to 88% yield). We found that β -aryl- and β -amino-substituted aldehydes are also suitable substrates (entries 20 to 22; 63 to 88% yield). Perhaps most remarkable is the capacity of this activation mode to enable bond formations at highly sterically congested centers, as highlighted with the β,β' -disubstituted aldehydes (entries 23 to 25). In each case, quaternary carbon-containing β -aryl products are forged with excellent levels of ef-

iciency (entries 23 to 25; 70 to 74% yield). These results are consistent with an early transition state in the critical radical-radical coupling (Fig. 2, **5** + **10**), which in turn would minimize the impact of nonbonding interactions. We recognized from an early stage that our transformation might allow direct β -arylation of propionaldehyde, thereby delivering a one-step approach to the production of hydrocinnamaldehyde equivalents. As revealed in entry 26, this strategy was indeed found to be feasible (78% yield).

As might be expected, the electronic nature of the β -enaminyll radical **10** plays a critical role in the efficiency of the arylation step. For example, β -aryl aldehyde substrates that are electron-

rich in nature, such as *p*-methoxyphenyl (entry 20) and *N*-methylindolyl (entry 21), readily undergo β -aryl coupling. In contrast, the use of 3-(*p*-cyanophenyl) propionaldehyde (the product of entry 26) results only in the recovery of starting materials, presumably due to the low nucleophilicity of the corresponding β -enaminyll radical. These results provide a mechanistic basis as to why mono-arylation adducts are selectively observed in this study, given the electron deficiency of the β -aryl products formed.

We next examined the scope of the aromatic coupling component in this synergistic catalysis protocol. As further shown in Fig. 3, a range of cyanobenzene and cyanoheteroaromatics have

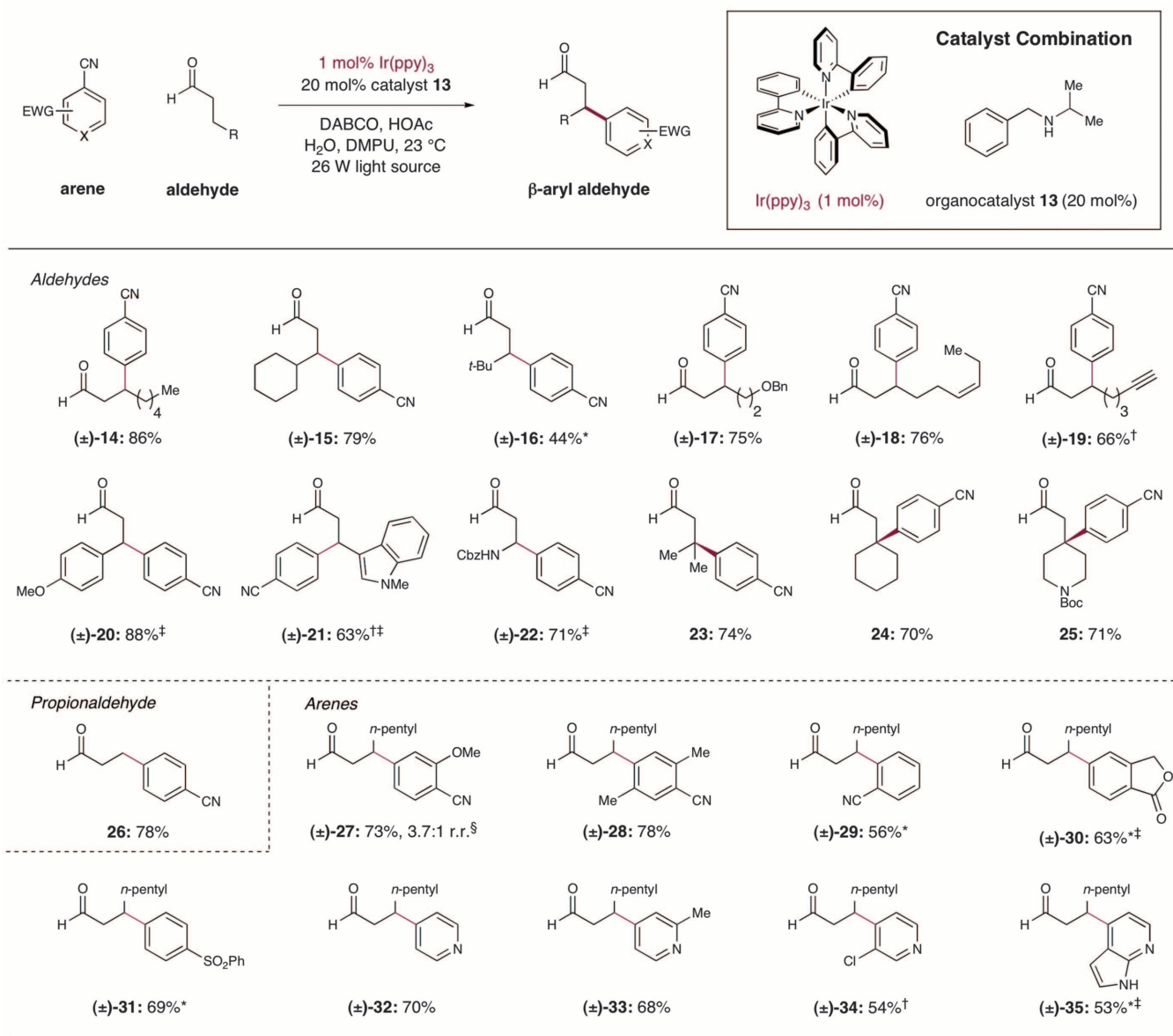


Fig. 3. Photoredox C–H β -arylation: aldehyde and arene scope. For each entry number (in boldface), data are reported as percent isolated yield. R = generic alkyl or aryl substituent; X = CH, C, or N; DMPU, 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one; DABCO, 1,4-diazabicyclo[2.2.2]octane; Me, methyl; Bn, benzyl; *t*-Bu, *tert*-butyl; Boc, *tert*-butyl carbamoyl; Cbz,

benzyl carbamoyl. *See supplementary materials for experimental details. †1.0 equiv of Na₂CO₃ added. ‡Product isolated as β -aryl alcohol. §Regiomer ratio (r.r.), determined by ¹H nuclear magnetic resonance analysis; see supplementary materials. Major isomer is shown; minor isomer is 3-methoxy-4-alkylbenzonitrile.

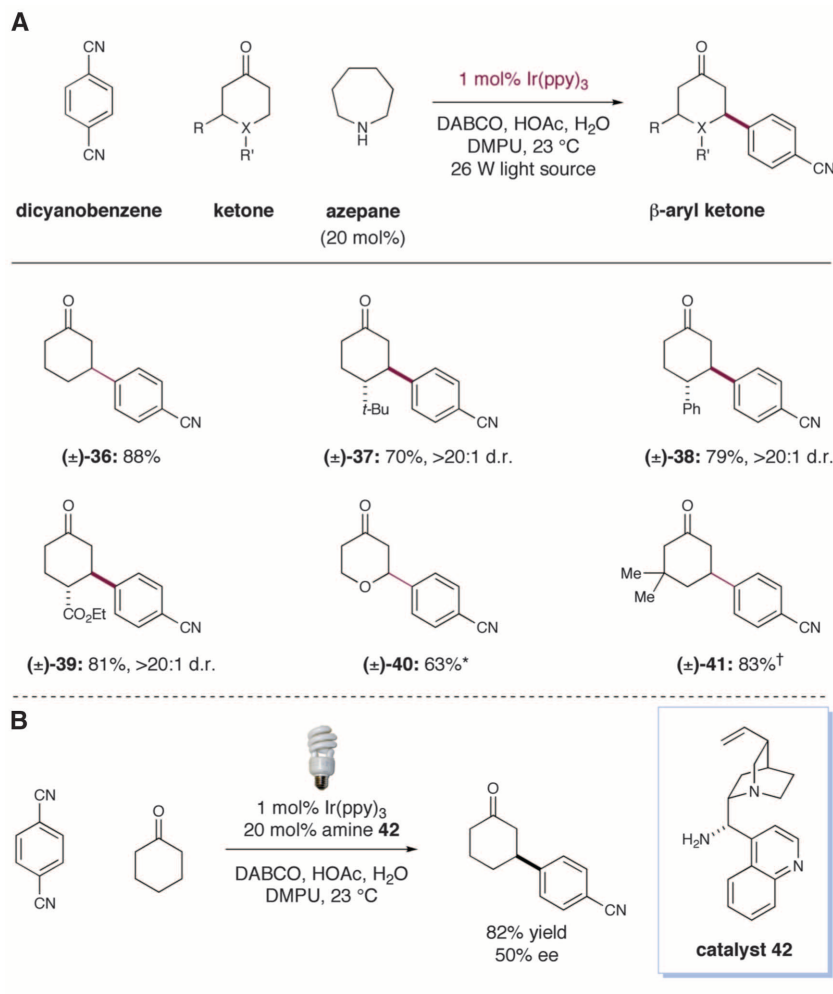


Fig. 4. (A) Direct β -arylation of ketones. For each entry number, data are reported as percent isolated yield; for entries 37 to 39, diastereomeric ratio (d.r.) was determined by ^1H nuclear magnetic resonance analysis. R = hydrogen or methyl; R' = hydrogen, generic alkyl, or aryl substituent; X = CH or O. (B) Enantioselective β -arylation of cyclohexanone (ee, enantiomeric excess). *20 mol % piperidine used as the organocatalyst. †40 mol % azepane used as the organocatalyst.

been found to be suitable substrates (Fig. 3, entries 27 to 35; 53 to 78% yield). Sterically hindered arenes, such as 2,5-dimethylterephthalonitrile, readily undergo addition to the activated 5π species **10** (entry 28, 78% yield). Moreover, cyanoaromatics that incorporate substituents at the ortho, meta, and para sites are readily tolerated (entries 27 to 30, 33, and 34; 54 to 78% yield). Although cyano-substitution appears to be essential for the arene coupling partner, superior yields are obtained when electron-withdrawing groups, such as esters and sulfones, are incorporated at the ortho and para positions (entries 29 to 31; 56 to 69% yield). With respect to heteroaromatic systems, a broad range of cyano-substituted pyridines with both electron-donating and electron-withdrawing substituents undergo β -coupling with good levels of efficiency (entries 32 to 35; 53 to 70% yield). Moreover, 7-azaindole, an important biological isostere for indole in medicinal chemistry, readily participates in this transform (entry 35). The formation of cyanide is an

unfortunate drawback of this protocol but can be easily washed away with an aqueous work-up.

Given that enamine formation is a central step in the formation of the 5π intermediate **10**, we presumed that ketones should also be amenable to this β -coupling reaction, provided that a suitable amine catalyst could be identified. As shown in Fig. 4, the seven-membered azepane system was found to be an exceptional catalyst for the β -functionalization of a myriad of cyclohexanone derivatives (Fig. 4A, entries 36 to 41; 63 to 88% yield). As was the case with aldehydes, this photoredox arylation protocol is tolerant of significant steric variation on the cyclohexyl ring (entries 37, 38, 39, and 41; 70 to 83% yield). Moreover, useful levels of trans-diastereocontrol are accomplished with ketone substrates bearing substituents at the 4-position (entries 37 to 39; >20:1 d.r., 70 to 81% yield). Heteroatom-containing ketones also serve as competent coupling partners (entry 40, 63% yield). The addition of increased quantities of water was

required with ketone substrates to avoid the production of bis-3,5- β -arylation products, presumably due to the need for a fast enamine hydrolysis step after the initial arylation. Preliminary studies have revealed that cinchona-derived organocatalysts, such as amine **42**, effect the β -arylation of cyclohexanone with promising levels of enantioselectivity (Fig. 4B, 82% yield, 50% ee). This result clearly demonstrates that this activation mode is amenable to asymmetric catalysis, and work on this topic is ongoing.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Fig. S1
Table S1
NMR Spectra
References (27–37)

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Direct Observations of the Evolution of Polar Cap Ionization Patches

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Patches of ionization are common in the polar ionosphere, where their motion and associated density gradients give variable disturbances to high-frequency (HF) radio communications, over-the-horizon radar location errors, and disruption and errors to satellite navigation and communication. Their formation and evolution are poorly understood, particularly under disturbed space weather conditions. We report direct observations of the full evolution of patches during a geomagnetic storm, including formation, polar cap entry, transpolar evolution, polar cap exit, and sunward return flow. Our observations show that modulation of nightside reconnection in the substorm cycle of the magnetosphere helps form the gaps between patches where steady convection would give a “tongue” of ionization (TOI).

Polar cap patches are islands of high number-density F region ionospheric plasma, poleward of the auroral oval, surrounded by plasma of half the density or less (1, 2). Previous theories and observations indicated that patches are formed by ionospheric dynamics in the “cusp region” (3–8), in which one mechanism considered to be dominant, at least in the European sector (2), is associated with magnetic reconnection between a (draped) southward interplanetary magnetic field (IMF) and the geomagnetic field at the dayside magnetopause. Patches follow the flow streamlines of the Dungey convection cycle (9), moving across the pole from the dayside to the nightside (10, 11), and have been seen exiting the polar cap and entering the nightside auroral oval (5, 12). It has long been recognized that mid-latitude ionospheric plasma, produced by solar extreme ultraviolet (EUV) radiation, provides a viable reservoir of source plasma, although in some cases densities may be further enhanced by precipitation of solar wind particles precipitating into the cusp ionosphere (8, 10, 13, 14). Flow into the polar cap is restricted to a convection “throat” that, like the cusp precipitation, extends along the line in the ionosphere that is the magnetic footprint of the magnetopause reconnection (14).

Sometimes the enhanced ionization entering the polar cap takes the form of a continuous “tongue” of ionization (TOI) (15), rather than discrete patches, and numerical simulations readily reproduce a TOI by convecting solar-EUV-enhanced plasma antisunward through the throat (16). There has been considerable debate about why a TOI is not the usual occurrence,

and a number of mechanisms that divide it up into patches have been proposed (4–6), with implications for a variety of operational systems (2, 17). In addition, the convection that causes the motion of patches is not usually steady; rather, it varies with the substorm cycle (9) as the magnetic field accumulates and is then released in

the tail of the magnetosphere (18), but the effect on patch evolution has not been observed. It has been particularly difficult to study the detailed motion and evolution of patches because of poor data coverage. Although there are two-dimensional (2D) trajectory analysis codes and 3D ionospheric assimilation techniques (17, 19), neither has provided complete information. Here, we present continuous monitoring of both the plasma density and flow in a large fraction of the Northern Hemisphere convection zone over a full convection cycle (4 hours, with time resolution of 5 min) by combining the observations of the total electron content (TEC) (20) from the large and dense array of Global Positioning System (GPS) receivers (21, 22) with the large-scale coverage of the flows provided by the Super Dual Auroral Radar Network (SuperDARN) radars using the Map Potential technique (23, 24).

On 24 September 2011, a coronal mass ejection (CME) emerged from an active region on the Sun. This reached Earth’s magnetopause 2 days later at 12:37 UT, giving an enhancement of solar wind dynamic pressure, P_{Dyn} . Geomagnetic indices reveal that this produced a major disturbance (a magnetic storm), the ap planetary geomagnetic

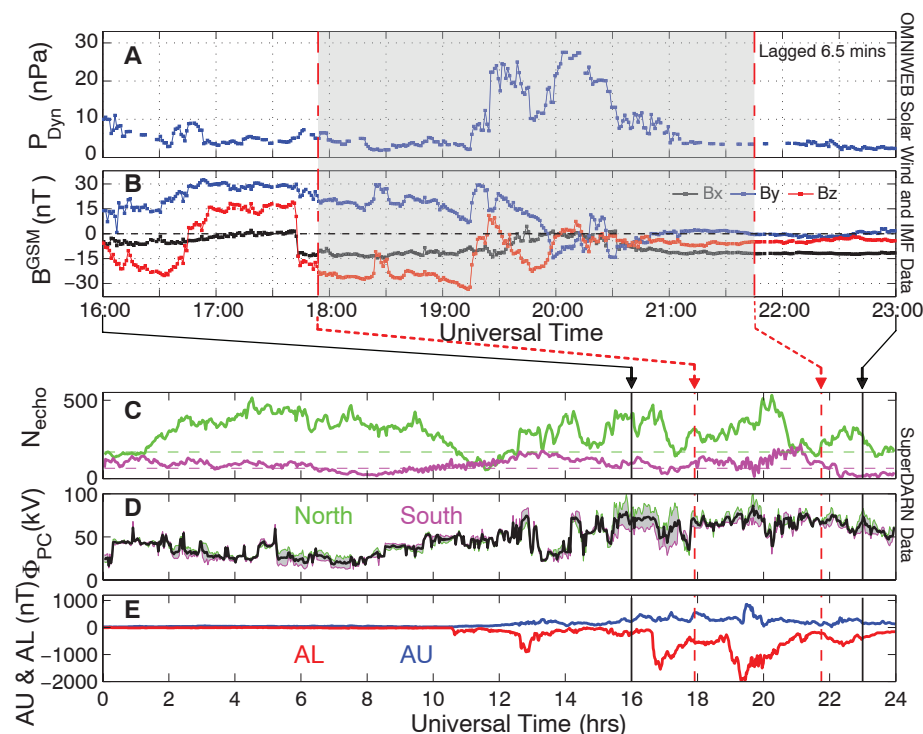


Fig. 1. An overview of the interplanetary conditions, the SuperDARN radar data, and the auroral electrojet (AE) indices on 26 September 2011. Parameters shown are (A) the solar wind dynamic pressure, P_{Dyn} ; (B) the IMF components in GSM coordinates; (C) the number of valid Doppler shift values N_{echo} in each 2-min convection pattern from the SuperDARN radar networks (green/mauve lines are for the Northern/Southern Hemispheres, and horizontal dashed lines are the averages for 1998 to 2012; for much of the period of interest, the SuperDARN data are in the top 20% in terms of quality indicated by N_{echo}); (D) the transpolar voltage Φ_{PC} from the SuperDARN radars (the black line is the average for the Northern and Southern Hemispheres, and the gray area gives the difference between the hemispheric values); (E) the provisional AE auroral electrojet indices from 11 stations: The blue line is AU (auroral upper) and the red line is AL (auroral lower). Interplanetary data have been lagged to the nose of the magnetosphere using THEMIS-A (Time History of Events and Macroscale Interactions during Substorms—A) observations (fig. S1).

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index (see supplementary materials) reaching 94 at 15 to 21 UT (a level exceeded less than 0.5% of the time). A second enhancement in P_{Dyn} impacted the magnetosphere at about 19:15 UT (Fig. 1A). There was a large and variable IMF at Earth, with two intervals of an exceptionally strong southward field (B_z , in red in Fig. 1B) ahead of this second P_{Dyn} pulse. This IMF orientation favors rapid reconnection at the dayside magnetopause and gave the growth phases of two substorms

that ended with the onset of their expansion phases at 16:35 and 18:54 UT [AL (auroral lower) subsequently falling to -1500 nT and -2000 nT, respectively (Fig. 1D)]. The presence of substorms reveals intervals of dominant magnetopause reconnection followed by dominant magnetotail reconnection, and polar ionospheric convection is then predicted by the expanding-contracting polar cap (ECPC) model (18). Numerical modeling of the response to an isolated pulse of southward

IMF using ECPC has shown that an initially localized convection enhancement on the day-side expands antisunward (14). However if, as in this case, a southward turning follows a previous one, a somewhat more global response of the convection pattern is expected (25) and, consistent with this modeling, the second southward turning of the IMF yielded an almost immediate rise in the transpolar voltage at 17:55 UT (Fig. 1D).

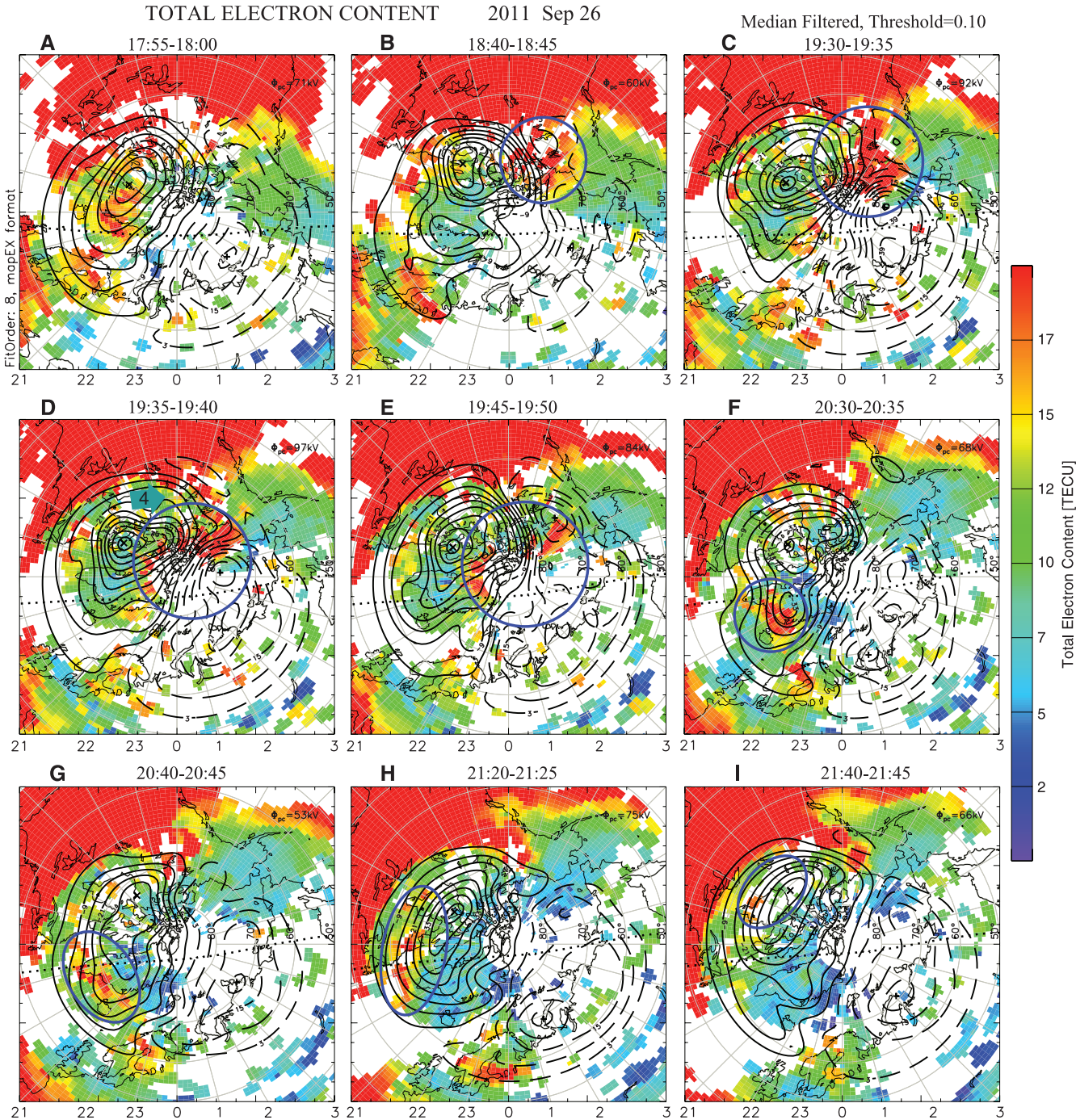


Fig. 2. (A to I) Extracts from a full series of 2D maps of median-filtered TEC (24) and ionospheric convection on a geomagnetic latitude/magnetic local time (MLT) grid with noon at the top (figs. S3 and S4 and movie S1). The dotted line across each panel is the day-night terminator at 100-km altitude. The blue circles and ellipses highlight the polar cap patch, the evolution of which is followed in this figure.

Figure 2 reveals the formation and evolution of a patch by mapping the TEC and flow streamlines. Figure 2A shows that the initial convection response was global, as discussed above, but as the IMF turned increasingly southward and the nightside reconnection voltage associated with the previous substorm decayed, the dominance of the dayside reconnection became apparent and the convection enhancement became more localized to the dayside (Fig. 2B) (14). After forming in the cusp region near noon (Fig. 2B), a large patch (ringed in blue) crossed through the throat, giving the local enhancement in TEC westward of the cusp. The strong positive IMF B_Y component implies westward flow in the throat; this has been invoked as a contributor to patch formation (4). The interval covered by Fig. 2, B and C, is the growth phase of the second substorm, during which the dayside polar cap boundary expanded equatorward (26), as predicted by the ECPC model. Such an expansion has been invoked as a cause of patch formation because the convection can move EUV-enhanced plasma toward the convection throat (5, 13). However, the convection data show that the expansion occurred around 18:45 UT (fig. S2), which is after the patch formation began. Hence the polar cap expansion contributed to the continued growth of this patch, but its initial growth was due to rapid convection through the throat of high-TEC flux tubes in the near-noon auroral oval (which, from earlier TEC maps, can be attributed to a

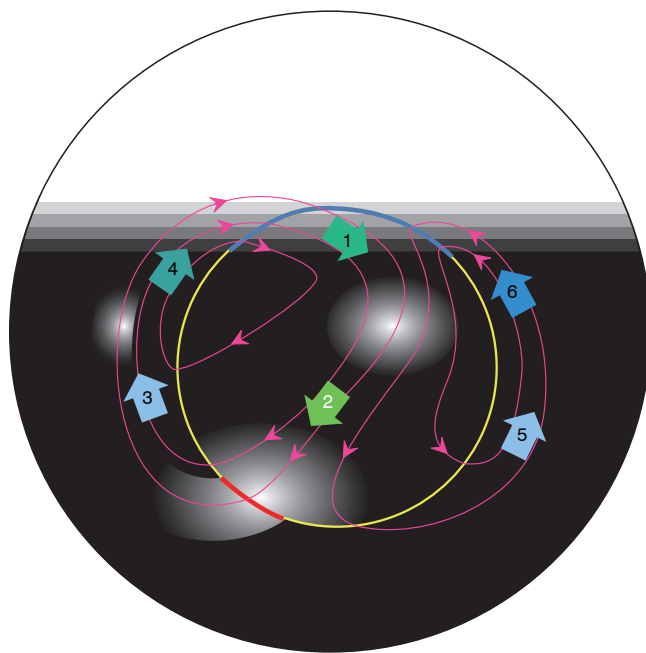
patch generated during the growth phase of the previous substorm that can be seen convecting sunward in the afternoon sector in Fig. 2A).

Interestingly, the patch seen forming in Fig. 2, B and C, did not directly move across the polar cap; rather, it was “stored” westward of the cusp region, where it grew in size. This is expected for a localized dayside convection enhancement, as seen in the SuperDARN convection patterns and as predicted by the ECPC model. Only when the convection enhancement expanded tailward (Fig. 2, D and E), did the patch migrate antisunward. The second substorm onset was between Fig. 2, B and C. (The patch appears to have bifurcated in Fig. 2E, but this is because there are no TEC data available poleward of 85°). The patch is completely separated from the high dayside TEC values by about 19:40 UT (Fig. 2E). It is useful to track back in time to define the evolution of the region of low TEC that “pinches off” the patch from the dayside: It can be seen to convect westward with the sunward return flow in the afternoon sector. Hence, these data answer the question of why this is a patch and not a TOI: It is pinched off by low TEC plasma in the post-noon auroral oval that is convected into the polar cap.

In Fig. 2, F to I, the patch is seen exiting the nightside auroral oval, breaking up into a number of plasma blobs, and moving sunward—as predicted in simulations—and returning sunward in the auroral oval of the dusk convection cell, which is consistent with the predictions from

previous theories and trajectory analysis techniques (16, 17). However, for the patch to exit the polar cap in this way requires ongoing nightside reconnection; without this, the open-closed boundary (OCB) would be “adiarctic” (Fig. 3), and the patch can only migrate slowly equatorward with the expanding polar cap boundary and, thus, would remain in the nightside polar cap (like the portion of the patch on the dusk convection cell in Fig. 3). Simulations show that the plasma recombination rates work on a time scale longer than the cycle time of plasma around polar cap cells, such that plasma concentrations convecting sunward in the auroral oval can be almost as large as in the newly formed patch in the dayside polar cap (16)—provided that tail reconnection allows the patch to exit the nightside polar cap. Nightside reconnection voltage dominates over dayside reconnection in substorm expansion phases, but is more restricted in rate and location (or even absent) during substorm growth phases (27). In addition, nightside reconnection may not be on the same cell (or even the streamlines within that cell) needed for reinjection into the polar cap in the cusp region. The ECPC model shows that, in a growth phase, the sunward-convecting regions are dominated by plasma that has been pushed sunward by the expanding polar cap but has not exited the polar cap through a reconnection site footprint. Much of this plasma could have been in the dark for extended periods and so may be of low density. Thus, nightside reconnection, or more specifically the lack of it, has a key role to play in forming the gaps between patches, and this can explain why those gaps are larger if substorm activity is absent (12) and why patches can be segmented even before they enter the cusp region (3).

Fig. 3. Schematic of the northern polar ionosphere during a substorm growth phase with southward IMF and $B_Y > 0$. Convection streamlines are in mauve. The boundary between open and closed field lines lies close to the poleward edge of the auroral oval: the blue/red OCB segments show where magnetic reconnection at the magnetopause/magnetotail is generating/destroying open flux in the Dungey convection cycle (9). In this case, magnetopause reconnection is dominant and the polar cap is expanding (18). The yellow OCB segments are “adiarctic” (meaning “not flowing across”) where flow streamlines cross the OCB because it is in motion and the plasma moves with it. The gray scale indicates plasma concentration, with white showing high values generated by solar EUV and black showing low values where plasma has decayed on the nightside. Convection leads to high-concentration plasma entering the polar cap from subauroral latitudes (arrow 1). The patches are transported antisunward across the polar cap (arrow 2) and evolve into “blobs”. These have been seen leaving the polar cap on the nightside, but they can only do so at locations that map to ongoing magnetotail reconnection. Hence, in this case, although blobs are seen in part of the auroral sunward return flow region on the dusk side (arrows 3 and 4), they are absent completely from the corresponding region on the dawn side (arrows 5 and 6).



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Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6127/1597/DC1
Supplementary Text
Figs. S1 to S4
Movie S1
References

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Structure of the Integral Membrane Protein CAAX Protease Ste24p

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Posttranslational lipidation provides critical modulation of the functions of some proteins. Isoprenoids (i.e., farnesyl or geranylgeranyl groups) are attached to cysteine residues in proteins containing C-terminal CAAX sequence motifs (where A is an aliphatic residue and X is any residue). Isoprenylation is followed by cleavage of the AAX amino acid residues and, in some cases, by additional proteolytic cuts. We determined the crystal structure of the CAAX protease Ste24p, a zinc metalloprotease catalyzing two proteolytic steps in the maturation of yeast mating pheromone **a**-factor. The Ste24p core structure is a ring of seven transmembrane helices enclosing a voluminous cavity containing the active site and substrate-binding groove. The cavity is accessible to the external milieu by means of gaps between splayed transmembrane helices. We hypothesize that cleavage proceeds by means of a processive mechanism of substrate insertion, translocation, and ejection.

Isoprenoid groups are conjugated to proteins by means of cysteine residues of CAAX acceptor sequences in which the cysteine attachment site is followed by two aliphatic amino acid residues and one unspecified residue at the protein C terminus. Isoprenylation is generally accompanied by two subsequent processing steps, proteolytic cleavage of the AAX residues and carboxymethylation of the newly exposed carbonyl group of the modified cysteine residue

(fig. S1). Some isoprenylated proteins also undergo additional proteolytic processing, including an additional cleavage by the same protease that initially removes the AAX residues. At least two classes of enzymes are responsible for the cleavage of isoprenylated proteins and peptides. One of these is the Ras-converting enzyme (Rce) family of type II prenyl proteases, responsible for proteolytic processing of signal-transducing proteins including Ras (1, 2) and the G_γ subunits of heterotrimeric GTP-binding protein (G protein) complexes (3). The other is the Ste24p family of type I prenyl proteases, first identified in yeast on the basis of its role in maturation of the mating pheromone **a**-factor (4–6). Extensive characterization of the role of Ste24p in **a**-factor processing has been conducted in the yeast system (7). Ste24p is localized to the endoplasmic reticulum membrane. Its proteolytic activity requires zinc, consistent with the fact that Ste24p contains the zinc metalloprotease signature motif HEXXH (8, 9).

A human ortholog of Ste24p, zinc metalloprotease STE24 (ZMPSTE24), can complement

the full function of yeast Ste24p (6). The only known substrate for ZMPSTE24 is prelamin A, the precursor to the nuclear intermediate filament protein lamin A. Lamins provide mechanical stability to the nuclear envelope, function as scaffolds for localization of other proteins and for cytoskeletal attachment, regulate chromatin, and are implicated in transcription and DNA repair and replication (10). Mutations in either ZMPSTE24 or the processing site of prelamin A are associated with a spectrum of premature-aging diseases referred to as progeria (11). The severity of different forms of progeria is reported to be correlated with extent of loss of ZMPSTE24 activity (12). Also, ZMPSTE24 (and *Saccharomyces cerevisiae* Ste24p) are inhibited by antiviral drugs designed to target the HIV aspartyl protease, and this off-target interaction may give rise to some of the severe side effects of these drugs (13, 14).

Ste24p from *Saccharomyces cerevisiae* (ScSte24p) has been overexpressed previously in *S. cerevisiae* cells and purified (9, 15). To identify forms of the protein with enhanced stability and suitability for crystallization, we cloned and purified orthologs from nine yeast species closely related to *S. cerevisiae*. *S. mikatae* Ste24p (SmSte24p) is 96% identical to ScSte24p and is 37% identical to *H. sapiens* ZMPSTE24 (fig. S2). Purified SmSte24p is enzymatically active (fig. S3), and we obtained crystals of this protein that diffracted anisotropically to 3.1 Å resolution (and isotropically to 3.9 Å resolution). After obtaining a native data set and proceeding to make selenomethionine-containing SmSte24p (16), we discovered that the Structural Genomics Consortium (SGC) had solved the structure of human ZMPSTE24. Because of SGC's open access policy, the coordinates were deposited in the Protein Data Bank (PDB) before publication [PDB: 4AW6 (17)]. Thus, we solved the structure of SmSte24p by a combination of molecular replacement (MR) and single-wavelength anomalous diffraction (SAD) of the bound catalytic zinc atoms.

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The combination of MR and experimental SAD dispersion phases, along with application of noncrystallographic symmetry and solvent-density modification, yielded interpretable electron density maps (fig. S4). The anisotropic diffraction data likely results from the high solvent content (~80%) of the crystal, as well as the marked asymmetry of crystal contacts (fig. S5). The anisotropic data between 3.9 and 3.1 Å resolution make up a substantial fraction of the entire data set used for structure determination and refinement and increase the total number of reflections by ~30% compared with the isotropic data. The structure was refined to R and R_{free} values of 0.270 and 0.293, respectively, with good stereochemistry (table S1). Structure determination included extensive use of omit maps and real-space correlation coefficients (figs. S4 and S6 and tables S2 to S4). The asymmetric unit contains two nearly identical SmSte24p protomers [all-atom root mean square deviation (RMSD) of 0.7 Å] arranged as an antiparallel dimer. The protein construct present in the crystal is the 461-residue full-length protein with eight additional residues that remain after cleavage of the C-terminal affinity tags. The refined structure of chain A consists of residues 9 to 106, 114 to 338, and 347 to 445; that of chain B consists of

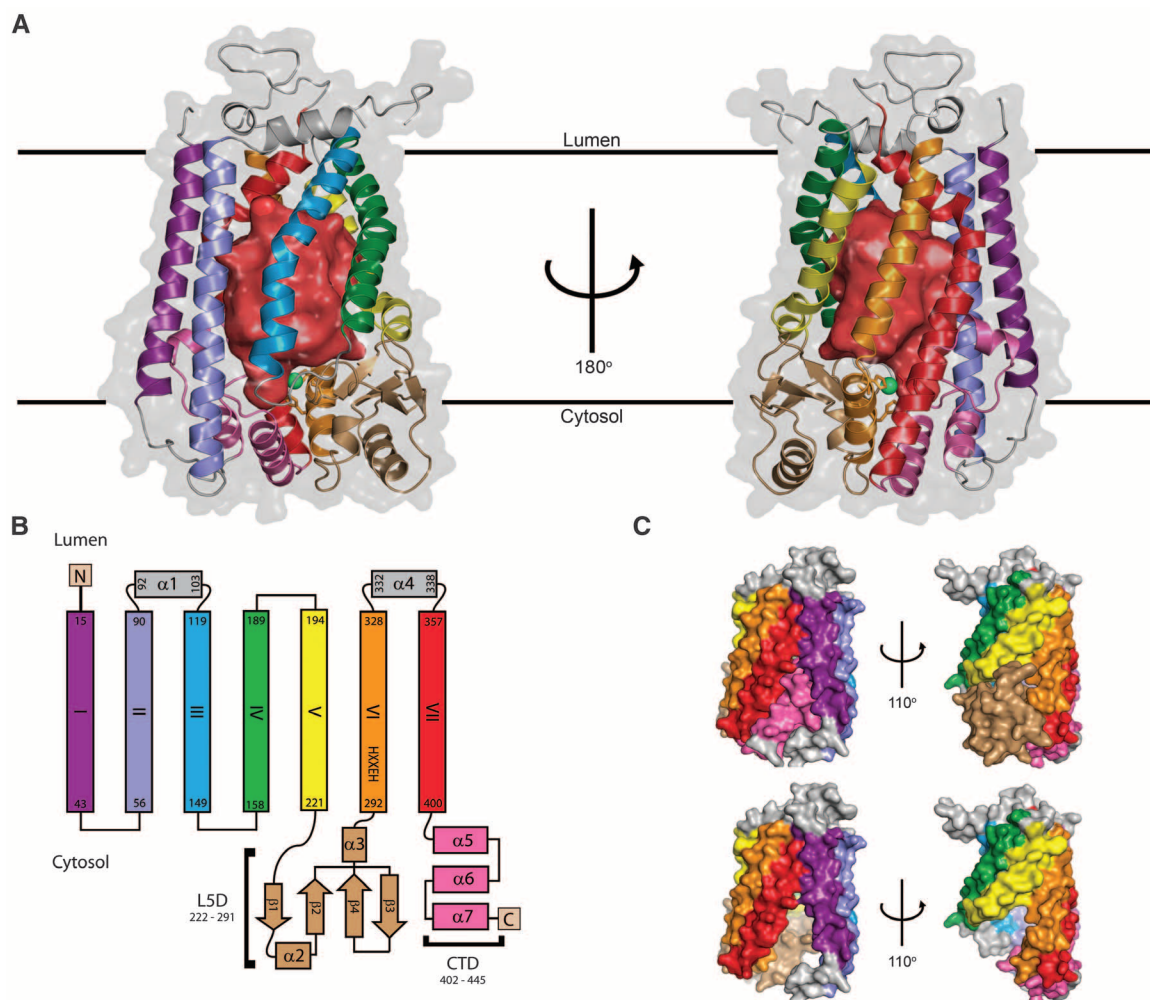
residues 11 to 103 and 109 to 446. The RMSD between SmSte24p and ZMPSTE24 is 1.2 Å.

SmSte24p is an integral membrane protein comprising seven transmembrane helices that surround a large cavity (~14,000 Å³) contained nearly completely within the membrane interior (Fig. 1A). The transmembrane helices range from 28 to 44 residues in length and form a fenestrated surface with gaps of up to 10 Å diameter between helices. The cavity is sufficient to contain a 10-kD protein or 450 water molecules. Helices III to VII are kinked or sharply curved, with helices III and IV containing proline residues at the sites of the kinks. Helix VI contains the zinc metalloprotease HEXXH motif. Two luminal loops, between helices II and III and between helices VI and VII, contain short α helices composed of 12 and 7 residues, respectively (Fig. 1B). These short helices, the other luminal loops, and the canted transmembrane helices combine to cap the luminal surface of the structure. The cytosolic-facing surface of the protein contains two additional domains: a 70-residue mixed α helix- β strand loop 5 domain (L5D) and a 44-residue α -helical C-terminal domain (CTD) (Fig. 1B). The L5D and CTD block the large gaps between helices V and VI and between helices VII and I, respec-

tively, and also cap the cytoplasmic surface of the structure (Fig. 1C).

Although no zinc was added during the expression, purification, or crystallization of SmSte24p, x-ray fluorescence scans of crystals clearly indicated a peak at the zinc edge, and diffraction data collected at this peak yielded a single prominent 5 σ anomalous difference Fourier peak in each protomer (Fig. 2A). The soluble protein thermolysin is the most thoroughly characterized zinc metalloprotease, with a large number of available crystal structures and extensive mechanistic analyses (18–22). Comparison of the active site of thermolysin to the zinc-binding site of SmSte24p reveals a striking structural congruence of nearly all of the residues responsible for zinc coordination, catalysis, and substrate recognition (Fig. 2). In particular, an eight-residue active-site substructure in thermolysin is nearly identical to the same substructure in Ste24p (Fig. 2C). Similarity between the active sites of thermolysin and an integral membrane protein zinc metalloprotease was previously noted for the archaeobacterial protein site-2 protease (S2P) (23). Ste24p and S2P have different topologies; also, unlike Ste24p, which processes peptides expected to reside at the membrane surface, S2P

Fig. 1. (A) Overall structure of SmSte24p. Ribbon diagram of Ste24p highlighting pronounced kinks in transmembrane helices III to VII. The red surface in the center of the protein represents the boundary of the ~14,000 Å³ interior cavity, calculated with CASTp (30), visualized and rendered with HOLLOW (31). The green sphere is the catalytic zinc atom. (B) Topology diagram of SmSte24p; α helices are represented by rectangles; β strands are represented by arrows. (C) Surface representation of SmSte24p showing the two sets of splayed helices. (Left) The protein with (top) and without (bottom) the CTD (pink) to highlight the opening formed by helices I and VII. (Right) SmSte24p with (top) and without (bottom) the L5D (wheat) to highlight the opening formed by helices V and VI.



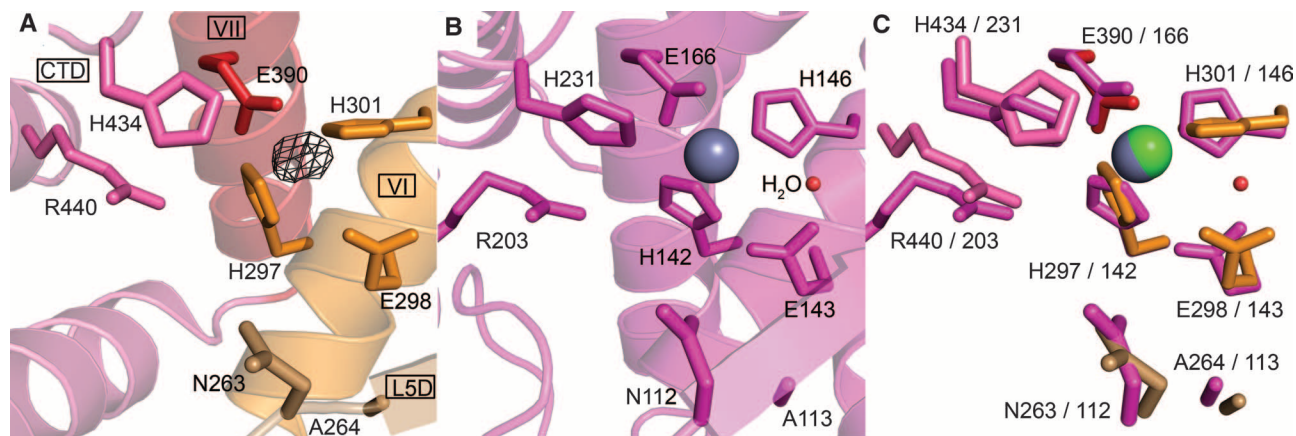


Fig. 2. Analysis of the SmSte24p active site. **(A)** The active site of SmSte24p contains the HEXXH motif in helix VI (H297–H301), the catalytic residue (E390), and substrate-coordinating residues N263, A264, H434, and R440. Mutation of H297, E298, or H301 diminishes Ste24p function (4, 32). The catalytic zinc atom is represented by anomalous electron difference den-

sity contoured at 5σ . **(B)** The active site of thermolysin (PDB:1LND) showing the HEXXH-motif (H142–H146), the catalytic residue (E166), and the substrate-coordinating residues N112, A113, R203, and H231. **(C)** Overlay of thermolysin and SmSte24p active sites showing their similarity (RMSD of overlaid residues: 0.5 Å).

catalyzes intramembrane proteolysis of trans-membrane proteins (24–26).

The interactions between Ste24p and its substrates that determine the specificity of the interaction remain poorly experimentally characterized; to date, only mutations immediately C-terminal to each of the cleavage sites in the *a*-factor precursor have been shown to affect cleavage efficiencies in *in vivo* studies (27, 28). To characterize a likely substrate-binding site in SmSte24p, we first produced a composite model of thermolysin with an occupied active site based on three structures of thermolysin in complex with substrates and inhibitors (Fig. 3A). We then generated an electron density envelope corresponding to the composite contents of the thermolysin active site (Fig. 3B) and mapped this onto the structure of SmSte24p by means of the same transformation matrix used to calculate the RMSD between the two enzymes' active-site residues. Finally, we fitted a farnesylated peptide fragment of the *a*-factor precursor (including its scissile bond) to this density (Fig. 3, C and D). The modeled position of the substrate places the farnesyl attachment site at a position adjacent to the internal cavity that affords ample volume for it to adopt a variety of conformations (Fig. 3, C and D). However, the modest resolution of our current structure precludes a detailed interpretation of this modeling.

The substrate-binding groove, formed by L5D and CTD, is contained at the cytoplasmic face of the internal transmembrane cavity of SmSte24p. The groove, nearly coplanar with the membrane surface and ~ 40 Å in length, traverses the cavity (Fig. 4, A and B). Each end of the groove is located between one of the pairs of splayed transmembrane helices (Fig. 1C). The high degree of similarity between the active sites of SmSte24p and thermolysin strongly suggests that the polarity (N-to-C direction) of peptide-protein substrates with respect to the active site is identical in the two enzymes. Thus, SmSte24p substrates

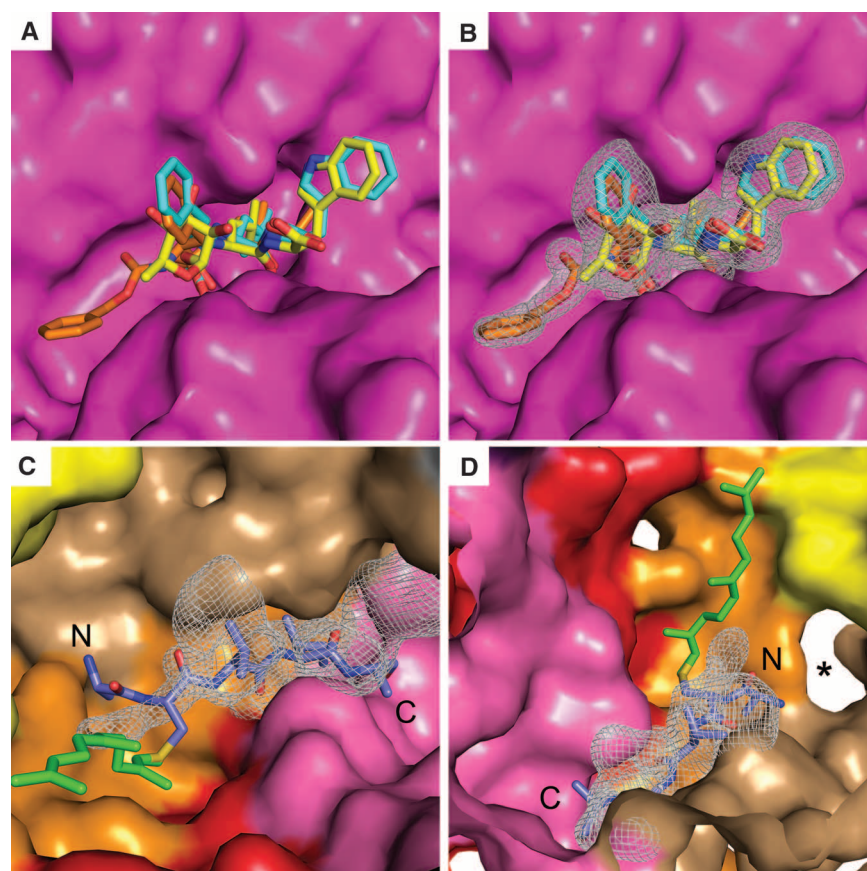


Fig. 3. Modeling of *a*-factor precursor binding to SmSte24. **(A)** Surface representation of the substrate-binding pocket of thermolysin with three different substrate/inhibitor molecules overlaid. **(B)** Electron density envelope calculated from (A). **(C)** Electron density envelope from (B) mapped onto the SmSte24p structure with an *a*-factor precursor peptide fragment (ACVIA) positioned within the density. **(D)** Alternate view of the modeled *a*-factor precursor peptide with attached farnesyl chain. The white area indicated by the asterisk identifies fenestration between helices V and VI.

can be expected to be oriented in the groove with their C termini pointing toward the portal between helices VII and I of Ste24p and their N termini

pointing toward the portal between helices V and VI (Fig. 4C). The localization of residues involved in enzymatic activity to the cytosolic-proximal

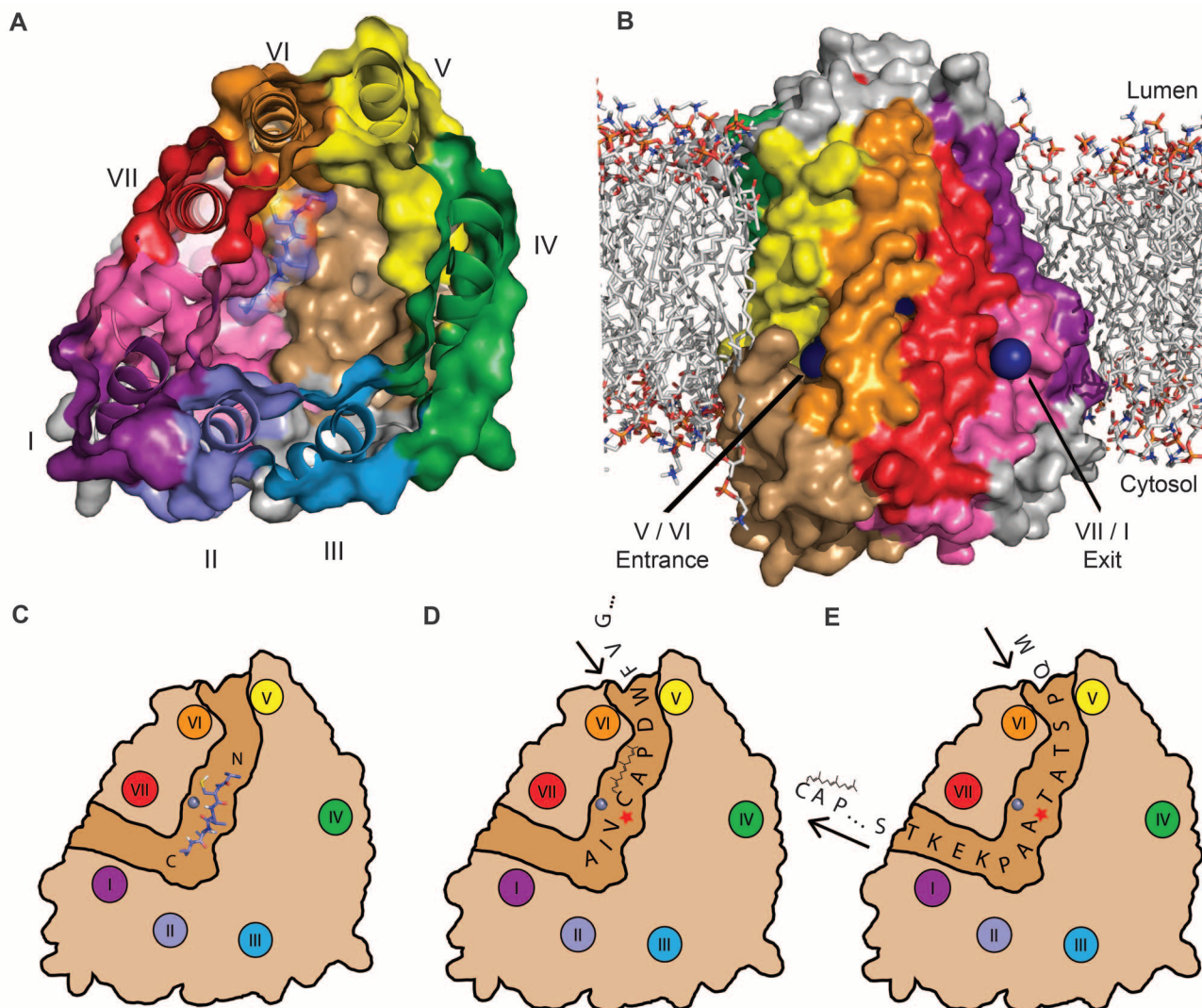


Fig. 4. Proposed Ste24p mechanism. **(A)** Cutaway view of the SmSte24p active site from the luminal surface. The modeled *a*-factor precursor peptide (from Fig. 3, C and D) is shown in blue. **(B)** Surface representation of SmSte24p positioned into a lipid bilayer model obtained from a published molecular dynamics simulation (33). Blue spheres denote gaps between helices V and VI and between helices VII and I that lead to the substrate-binding groove and internal active site contained within the internal transmembrane cavity (Fig. 1A).

(C) Schematic representation, to scale, of the continuous groove that traverses the cavity [determined with MOLE 2.0 (34)]. The ~40 Å tunnel can accommodate ~13 residues of substrate. **(D)** Insertion of the *a*-factor precursor C terminus into the entrance portal formed by helices V and VI. Cleavage by SmSte24p occurs at the CAAX motif indicated by the red star. **(E)** Subsequent substrate translocation, egress through the VII to I portal, and positioning of the second cleavage site.

portion of the cavity is reflected in higher sequence identity and conservation between SmSte24p and ZMPSTE24 in this part of the cavity (fig. S7).

These observations, together with previously reported functional similarity between Ste24p and ZMPSTE24, particularly the ability of ZMPSTE24 to fully complement a deletion of *STE24* in processing yeast *a*-factor (6), suggest a possible pathway for substrate processing by CAAX proteases. In considering a small substrate such as the *a*-factor precursor (36 residues in length), substrate binding could occur by entry of either its C or N terminus into the binding groove. However, prelamin A contains a folded domain of 647 residues N-terminal to both ZMPSTE24 cleavage sites (fig. S1A). Because it is unlikely that such a large domain could gain

access to the active site, the most likely pathway for entry of this substrate (and, by inference, all the substrates of this type of enzyme) into the active site is by means of insertion of its C terminus (Fig. 4D). The second proteolytic cleavages of the *a*-factor precursor and prelamin A could occur through processive further extension of the substrate C terminus into the substrate groove or cavity after the first cleavage (Fig. 4E). However, the presence of the large folded domain of ZMPSTE24 dictates that withdrawal of the final N-terminal cleavage products would have to occur by means of reversal of the initial route of entry. Because LSD and CTD block the gaps between helices V and VI and helices VII and I, respectively (Fig. 1C), movement of one or both domains may be required for substrate

processing. However, relatively modest conformational changes or movement may suffice to enlarge the fenestrations that already exist in these structural regions (Fig. 3D). Note that most of the missense mutations in human ZMPSTE24 that result in progeria map to the putative entrance and exit portals (fig. S8).

A possible role for the voluminous internal cavity is suggested by the consumption of a water molecule in each turnover of proteolytic enzymes. Although biological membranes are water-permeable, the dwell-time of water in the membrane interior is low, and maximizing the effective concentration of water may promote the proteolytic cleavage reaction by mass action. Thus, the cavity may serve as a water reservoir to facilitate CAAX protease substrate processing.

Other integral membrane proteases appear to rely on different mechanisms for obtaining access to water. The catalytic serine of rhomboid proteases is positioned at the base of a funnel-shaped periplasmic-accessible cavity that may be gated (29). The zinc atom of S2P is accessible to the cytoplasm by means of a narrow water-permeable channel present in the closed (to substrate) conformation of the enzyme (23). The role of the large cavity, the mechanism of specific recognition of cleavage sites with divergent sequences, the unusual dual-cleavage process, and the possible role of the farnesyl group in recognition are some of the questions raised by this provocative structure.

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The Structural Basis of ZMPSTE24-Dependent Laminopathies

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Mutations in the nuclear membrane zinc metalloprotease ZMPSTE24 lead to diseases of lamin processing (laminopathies), such as the premature aging disease progeria and metabolic disorders. ZMPSTE24 processes prelamin A, a component of the nuclear lamina intermediate filaments, by cleaving it at two sites. Failure of this processing results in accumulation of farnesylated, membrane-associated prelamin A. The 3.4 angstrom crystal structure of human ZMPSTE24 has a seven transmembrane α -helical barrel structure, surrounding a large, water-filled, intramembrane chamber, capped by a zinc metalloprotease domain with the catalytic site facing into the chamber. The 3.8 angstrom structure of a complex with a CSIM tetrapeptide showed that the mode of binding of the substrate resembles that of an insect metalloprotease inhibitor in thermolysin. Laminopathy-associated mutations predicted to reduce ZMPSTE24 activity map to the zinc metalloprotease peptide-binding site and to the bottom of the chamber.

The nuclear lamina is composed of intermediate filaments that give structure to the nuclei of cells in many multicellular organisms (1, 2). ZMPSTE24 (also known as

farnesylated-protein converting enzyme 1, FACE-1, Hs Ste24) is an inner nuclear membrane zinc metalloprotease that is essential for the maturation of lamin A, one of three lamin proteins found in the lamina of differentiated cells (3–5). Lamin A and B precursor proteins undergo a cascade of C-terminal, posttranslational modifications initiated by farnesylation of the cysteine on the C-terminal CAAX motif (where A is any aliphatic residue and X is any residue). ZMPSTE24 or the Rce1 protease (6) subsequently cleave

the AAX residues from the C terminus of prelamin A (the CAAX cleavage reaction), followed by carboxymethylation of the farnesyl cysteine. ZMPSTE24 then cleaves an additional 15 residues from the C terminus of prelamin A. This removes the membrane-embedded farnesyl cysteine (fCys) and releases mature lamin A into the nucleoplasm (5). The ZMPSTE24 yeast homolog, Ste24, performs two similar cleavage reactions on the yeast mating pheromone α -factor (7).

Mutations in either prelamin A or ZMPSTE24 cause a family of diseases called laminopathies, which result from accumulation of farnesylated, unprocessed prelamin A in the nucleus. ZMPSTE24-null mice have spontaneous bone fractures, cardiomyopathy, severe muscle weakness, and hair loss (3, 8). In humans, the severity of ZMPSTE24-dependent laminopathies correlates with the level of residual protease activity (9). The most severe ZMPSTE24-dependent laminopathies are neonatal lethal restrictive dermopathy (RD) (4, 10–12) and the premature aging (progeria) disease atypical Hutchinson-Gilford progeria syndrome (HGPS) (13). Milder laminopathies—such as mandibuloacral dysplasia type B (MAD-B) (14) and, potentially, metabolic syndrome (MS) (15)—have a range of symptoms including lipodystrophy (LD) and insulin resistance (16), bone and skin abnormalities, and cardiomyopathy (14, 15, 17). During normal aging, ZMPSTE24 expression decreases in vascular smooth muscle cells, which leads to accumulation of unprocessed prelamin A, disrupting

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mitosis and DNA damage repair (18, 19). There is also evidence that ZMPSTE24 is inhibited by certain HIV protease inhibitors, and the side effects of taking these drugs resemble laminopathies (20).

We solved the 3.4 Å structure of human ZMPSTE24 to understand how this enzyme cleaves prelamin A and how mutations in ZMPSTE24 cause laminopathies. The structure was determined from protein purified in two different detergent-lipid systems (fig. S1 and table S1), which gave two independent crystal packing arrangements, each with four copies of the structure in the asymmetric unit. Phases were obtained from an ethylmercury thiosalicylate derivative and eightfold averaging (fig. S2 and table S2) (21).

ZMPSTE24 adopts a seven transmembrane α -helical barrel structure (Fig. 1, A and B), enclosing an unusually large chamber (12000 Å³; 40 Å deep by 25 Å wide), that spans the membrane (Fig. 1C). On the nucleoplasmic side of the membrane, the chamber is sealed by a M48 zinc metalloprotease domain (22) that is reminiscent of thermolysin (23) and Q74D82 peptidase [Protein Data Bank (PDB) accession no. 3c37] (24) (fig. S3). The seven transmembrane helices (TMHs) are packed in an antiparallel α -helical bundle (Fig. 1B), with the zinc metalloprotease fold inserted between TMH5 and TMH6 (Fig. 1B and fig. S3). On the endoplasmic reticulum (ER) luminal face of the membrane, the bottom of

the chamber is sealed by three luminal (L) α helices, LH1, LH2 and TMH7A. There are four fenestrations allowing access to the chamber from the nucleoplasm and the membrane (Fig. 1C and fig. S4). Molecular dynamics simulations of ZMPSTE24 in lipid bilayers showed that the structure is stable when the chamber is filled with water (fig. S5). The interior of the chamber has a mixed polar-nonpolar character with a prominent charged patch between TMH1 and TMH7 and three hydrophobic patches on the adjacent wall between helices TMH4 and TMH5, around the extensive S₁' and S₂' substrate specificity sites, and on the ER luminal face of the chamber (Fig. 1C and fig. S4).

Using a synthetic peptide substrate that matches the C terminus of prelamin A (Ser⁶³⁶ to fCys⁶⁶¹), we showed that purified ZMPSTE24 has the expected proteolytic activity at the second cleavage site (Fig. 1D and fig. S6) (21). Shorter peptides (<15 residues), representing the first and second cleavage sites, were incorrectly cleaved at sites adjacent to the expected cleavage, in addition to the expected reactions (Fig. 1, D to F, and fig. S6), consistent with ZMPSTE24's requiring a longer substrate, with the farnesyl group attached, for accurate alignment of the peptide in the active site, as previously reported (25).

The catalytic site of ZMPSTE24 lies at the apex of the chamber, on the nucleoplasmic side of the membrane (Fig. 1A). The zinc ion is coordinated by His³³⁵ and His³³⁹ from the HEXXH zinc metalloprotease motif (H³³⁵ELGH in ZMPSTE24) and Glu⁴¹⁵ from TMH7 (Fig. 2A and fig. S3A). By analogy with other zinc metalloproteases, Glu³³⁶ from the HEXXH motif is predicted to be the catalytic residue, aligning and activating the catalytic water molecule, which attacks the substrate (fig. S7A). Mutation of this residue to alanine caused a reduction in ZMPSTE24 activity to one-fifth that with glutamic acid (fig. S6). Structural alignments predict that Asn²⁶⁵ and His⁴⁵⁹ would stabilize the transition state during catalysis (fig. S7).

The potential substrate-binding site was inferred from a 3.8 Å structure of a complex between ZMPSTE24 and a Cys-Ser-Ile-Met (CSIM) tetrapeptide from the C terminus of prelamin A (Fig. 2, B and C, and fig. S8). The binding mode is similar to that of an insect metalloprotease inhibitor in thermolysin (PDB 3ssb) (26), although, in ZMPSTE24, the tetrapeptide is translated by one residue relative to the expected binding mode (fig. S8). This is consistent with the activity data obtained for shorter peptides, where cleavage occurs between Ser⁶⁶² and Ile⁶⁶³ (Fig. 1F). The peptide-binding groove lies between the Zn²⁺ ion and strand β 3 of the metalloprotease β sheet and potentially interacts with highly conserved residues (fig. S9), such as Asn²⁶⁵ on β 3, His⁴⁵⁹ on a loop between metalloprotease helices (MHs) MH5 and MH6, and Arg⁴⁶⁵ on MH6. There is a deep pocket encompassing both the S₁' and S₂' subsites (Fig. 2B), which is lined by highly conserved hydrophobic residues contributed by MH3, MH4,

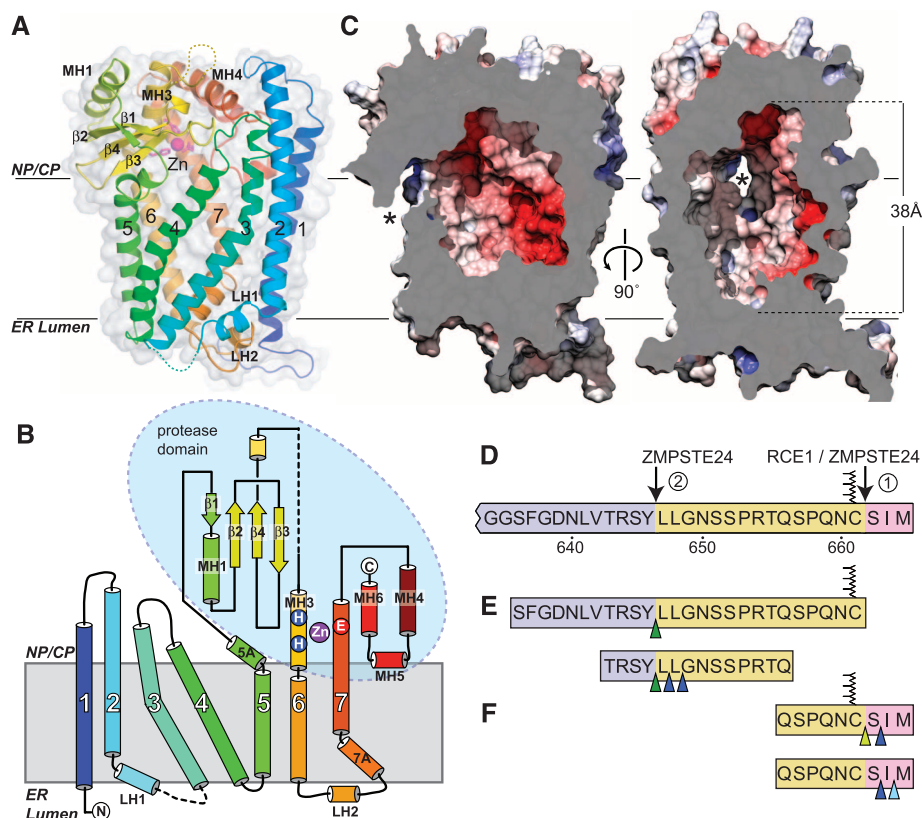


Fig. 1. Structure and activity of the ZMPSTE24 zinc metalloprotease. **(A)** Cartoon representation of the structure, viewed in the plane of the membrane. The structure is colored from blue to red from N to C terminus. The catalytic zinc and its coordinating residues are highlighted in magenta. Horizontal lines indicate the approximate extent of the lipid bilayer as observed in MD simulations. The nucleoplasmic-cytoplasmic (labeled NP/CP) and ER lumen sides of the membrane are indicated. **(B)** Topology diagram highlighting the arrangement of TMHs, colored as in (A). The metalloprotease fold is highlighted in pale blue. **(C)** Cut-away molecular surface representations highlighting the intramembrane chamber. The surface is colored according to electrostatic potential [contoured from -10 kT/e (red) to $+10$ kT/e (blue)] and sliced (gray) perpendicular to the plane of the membrane. The left image is in the same orientation as in (A) and the two surfaces show perpendicular views. The location of a fenestration between TMH5 and TMH6 in the chamber wall is marked with an asterisk. **(D)** ZMPSTE24 is thought to cleave the C terminus of prelamin A [sequence shown in (D)] at the two sites indicated by black arrows. **(E)** Cleavage was assessed using synthetic peptides derived from the C terminus of prelamin A with a mass spectrometric assay (21) (fig. S5). Two peptides (26-amino acid peptide including fCys⁶⁶¹ and an 11-amino acid peptide) probing the second cleavage site were cleaved between Tyr⁶⁴⁶ and Leu⁶⁴⁷, as expected. Additional cleavages were observed for the shorter peptide between Leu⁶⁴⁷ and Leu⁶⁴⁸ and between Leu⁶⁴⁸ and Gly⁶⁴⁹. **(F)** Two 9-amino acid peptides, with and without a fCys⁶⁶¹ were unexpectedly cleaved between Ser⁶⁶² and Ile⁶⁶³. A small amount of the expected product (Cys⁶⁶¹-Ser⁶⁶²) was observed when the peptide was farnesylated, and a small amount from an Ile⁶⁶³-Met⁶⁶⁴ cleavage was obtained with the unfarnesylated peptide. Green and blue triangles indicate expected and additional cleavages, with light colors for low-abundance reactions.

and MH6. These hydrophobic pockets could dictate the specificity of substrate binding for residues adjacent to the cleavage sites, by analogy with other thermolysin-like metalloproteases.

The prelam A substrate could enter the chamber via one of the fenestrations in the chamber wall (Fig. 1C and fig. S4). The chamber is too small to accommodate the whole of the 74-kD prelam A. Instead, the unstructured C terminus would insert into the chamber, entering and leaving by the same route. The fenestration between TMH5, TMH6, and the β hairpin connecting β 3 and β 4 (Figs. 1C, 2B, and 3A and fig. S4) is the most likely entry route. It connects the chamber with the nucleoplasm and the inner leaflet of the inner nuclear membrane, the locations of the protein and farnesyl moieties of the substrate (Fig. 3, A and B). This entry route would provide the correct alignment of the prelam A C terminus with the active site of ZMPSTE24 for cleavage of the SIM sequence. Once cleaved, the tripeptide product could exit via a fenestration between TMHs 1 and 2 (Fig. 2B and fig. S4). The partially processed prelam A C terminus would then leave the chamber completely or project into the membrane through one of the fenestrations, which would allow carboxymethylation by isoprenylcysteine carboxyl methyltransferase (ICMT), before prelam A reenters the chamber. The second cleavage site is 15 residues from the farnesylated Cys⁶⁶¹ at the C terminus. The chamber has sufficient space for these additional residues and potentially provides a binding site for the farnesyl group at the ER lumen end of the chamber, which aligns the substrate for the second cleavage (Fig. 3B). The C terminus of lamin A would then leave the chamber as soluble nucleoplasmic lamin A. Further mutagenesis and protein-peptide complex structures are needed to define the exact interactions between the protein and the substrates during these complex reactions.

Laminopathies caused by ZMPSTE24 mutations are recessive. The more severe diseases (RD and HGPS) have mutations that cause complete loss of function of both alleles, whereas the milder diseases (MAD-B, LD, and MS) have one or two alleles with partial activity (9). Inactivating mutations are caused by truncations, deletions, or frameshift mutations (fig. S10). The structure suggests that all these mutations are likely to result in improperly folded, inactive protein. There are also six disease-causing point mutations that reduce ZMPSTE24 activity. Five are mutations of highly conserved residues found in the zinc metalloprotease domain (N265S, L438F, L462R, W340R, and P248L) (Fig. 4, A and B), and the other mutation (L94P) is found at the ER lumen end of the barrel and is not conserved (Fig. 4, A and C).

The N265S point mutation causes MAD-B (27, 28) or atypical HGPS (29). In both cases, one allele is truncated and the other has the N265S mutation, which causes a reduction in specific activity of ZMPSTE24 to 5% of that

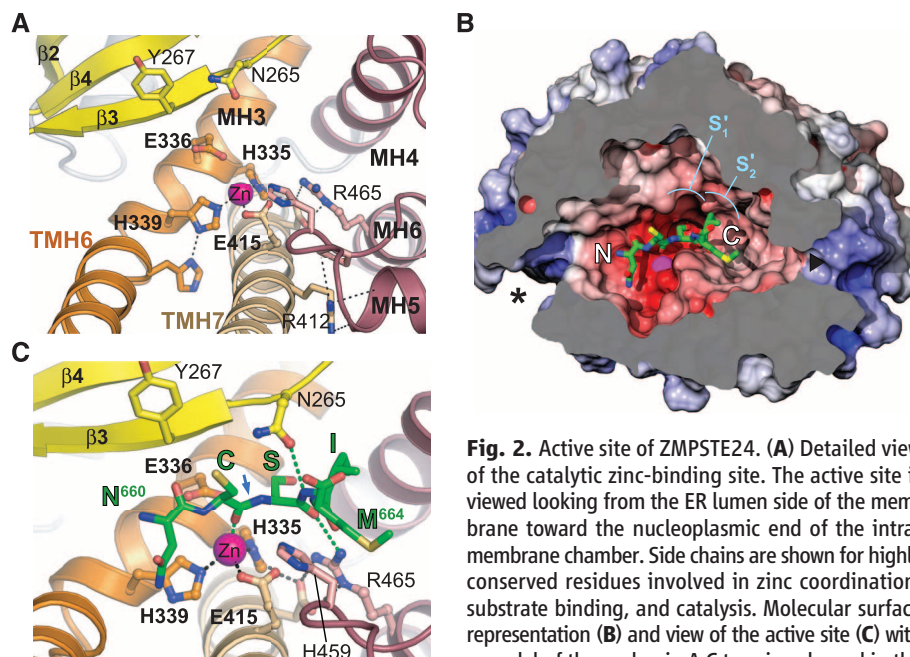


Fig. 2. Active site of ZMPSTE24. (A) Detailed view of the catalytic zinc-binding site. The active site is viewed looking from the ER lumen side of the membrane toward the nucleoplasmic end of the intramembrane chamber. Side chains are shown for highly conserved residues involved in zinc coordination, substrate binding, and catalysis. Molecular surface representation (B) and view of the active site (C) with a model of the prelam A C terminus bound in the active site, viewed from inside the chamber. The peptide binding model was obtained by using both experimental electron density from a 3.8 Å structure of the E336A mutant of ZMPSTE24 in complex with Cys⁶⁶¹-Met⁶⁶⁴ prelam A peptide and knowledge of the binding mode of peptide inhibitors to thermolysin (fig. S8 for more detail). Peptide carbons are colored green. (B) is colored as for Fig. 1C and (C) as for (A). The fenestration, which could act as the entry and exit route for the prelam A C terminus, is marked with an asterisk, and the potential exit route for the SIM product is shown with a black triangle. The expected cleavage site is indicated by a blue arrow in (C).

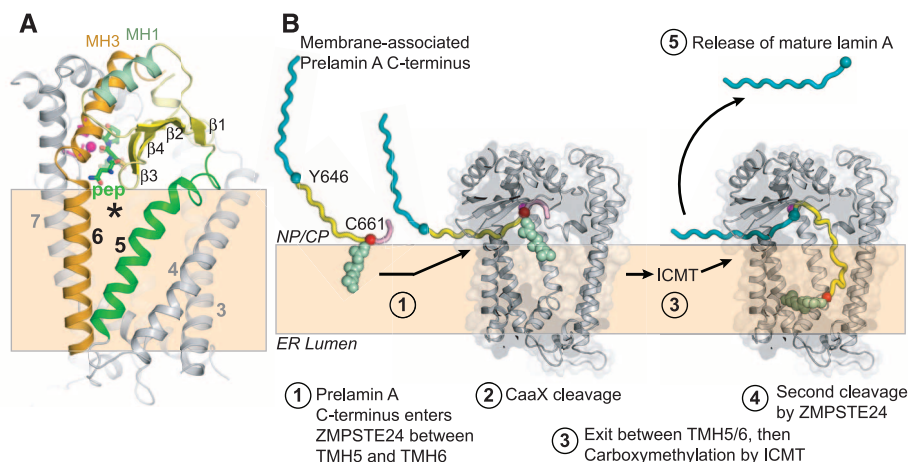


Fig. 3. Proposed substrate entry route for the C terminus of prelam A and scheme for the reactions performed by ZMPSTE24. (A) The fenestration between TMH5 and TMH6, below the β 3- β 4 hairpin is likely to be the entry route for the substrate. It provides a direct route into the active site, such that the C terminus of prelam A would be aligned with the peptide binding site identified in our protein-peptide complex. The modeled peptide is shown with the carbon atoms in green. (B) Scheme for the reactions performed by ZMPSTE24. The C terminus of prelam A is colored as in Fig. 1D, with the farnesyl group shown in pale green and the residues preceding the cleavage sites highlighted. Steps in the reaction scheme are numbered.

without the mutation (9). This mutation is likely to interfere directly with catalysis. Asn²⁶⁵ lies in the substrate-binding and catalytic site (Figs. 2A and 4B). It occupies a position equivalent to Asn¹¹² in thermolysin and is likely to fulfill the same function, that is, stabilizing the transition state intermediate by accepting a hydrogen bond from the N atom of the scissile bond (fig. S7).

Two point mutations in the zinc metalloprotease domain, L438F and L462R, lie adjacent to the peptide binding site, lining the extended S₁'-S₂' subsite (Figs. 2B and 4B). The L438F mutation was observed in a patient with MS (15) and causes a reduction in specific activity to 1% of that without the mutation. The L462R mutation causes the much more severe RD (30) (there

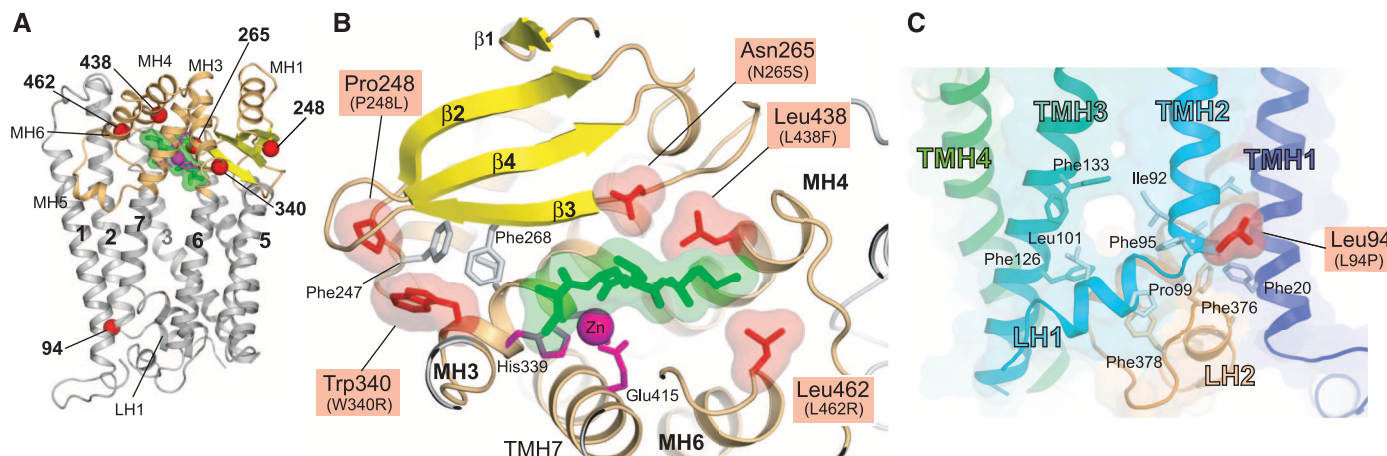


Fig. 4. Laminopathy-causing mutations in ZMPSTE24. Six mutations in ZMPSTE24 lead to reduced enzyme activity and varying severity of disease. **(A)** Five of these mutations (N265S, L438F, L462R, W340R, and P248L) lie in the zinc metalloprotease domain (gold) and one (L94P) is found in the ER lumen end of the chamber. The model for the bound CSIM tetrapeptide is shown in green. **(B)** Three mutations (N265S, L438F, and L462R) cluster around the

substrate-binding or active site, and two MAD-B mutation sites (W340R and P248L) are located near the β 3- β 4 hairpin adjacent to the proposed substrate entry fenestration. **(C)** The L94P mutation lies at the elbow between TMH2 and LH1. These two helices, together with TMH3 pack together to form a fenestration, adjacent to Phe¹³³, that links the chamber to the lipid bilayer. Mutated residues are shown as red sticks with semi-transparent molecular surfaces (B and C).

are no activity data available for this mutation). Both mutations are likely to affect peptide binding because of the insertion of a large side chain into the peptide binding site. A bulky, charged arginine in this site is likely to disrupt the packing of MH4 and MH6 and could potentially displace the associated, conserved catalytic residue His⁴⁵⁹ (equivalent to His²³¹ in thermolysin), which is thought to stabilize the transition state.

The W340R mutation causes MAD-B (12, 14) and a reduction in specific activity to one-sixth of that without the mutation (9). Trp³⁴⁰ lies at the C-terminal end of the MH3 HEXXH-containing helix, where it forms part of a cluster of hydrophobic residues (including Leu³³⁷, Phe²⁴⁷, and Phe²⁶⁸) that positions the MH3 helix and the Zn²⁺-binding histidines in the active site. The W340R mutation is likely to affect the position of the β 3- β 4 hairpin above the putative substrate entry route. Another mutation that leads to MAD-B (P248L) (31, 32) is also adjacent to Trp³⁴⁰ and the β 3- β 4 hairpin, forming a tight turn between MH1 and strand β 2 (Fig. 4B). Replacement of proline with leucine is likely to disrupt the tight turn and also to displace the β hairpin. The P248L mutation is also predicted to disrupt the hydrophobic cluster behind the HEXXH helix.

The L94P point mutation, found in a case of MAD-B (17), results in an almost complete loss of protease activity (9). Leu⁹⁴ is located at the bottom of the barrel, at the site of an elbow joint between TMH2 and LH1 (Fig. 4, A and C). The Leu⁹⁴ side chain points into the lipid bilayer, adjacent to a cluster of hydrophobic residues (Ile⁹², Leu⁹³, Phe⁹⁵, Ile⁹⁸, Pro⁹⁹, Leu¹⁰¹, Phe¹²⁶, and Phe¹³³), which line a fenestration that links the ER lumen end of the chamber with the lipid bilayer. The L94P mutation may disrupt the elbow joint between the helices, destabilizing the protein fold, consistent with the observation that this mutant expressed at much lower levels in yeast cells

(9). This region of the protein might also play a role in substrate binding or product release.

The ZMPSTE24 crystal structures provide a structural basis for protease-associated laminopathies. The known mutations are predicted to exert their effects through a variety of mechanisms: directly on catalysis, by affecting substrate binding, or by disrupting helix packing. There is still much to be learned about this medically important protein, such as how the substrate enters the protein, precisely how it recognizes both cleavage sites while avoiding cleaving other farnesylated proteins, where the farnesyl group binds during the first and second cleavage reactions, and why ZMPSTE24 needs such a large, hydrophilic chamber spanning the nuclear membrane in order to cleave prelamin A.

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Supplementary Materials

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Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance

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The diversity and abundance of wild insect pollinators have declined in many agricultural landscapes. Whether such declines reduce crop yields, or are mitigated by managed pollinators such as honey bees, is unclear. We found universally positive associations of fruit set with flower visitation by wild insects in 41 crop systems worldwide. In contrast, fruit set increased significantly with flower visitation by honey bees in only 14% of the systems surveyed. Overall, wild insects pollinated crops more effectively; an increase in wild insect visitation enhanced fruit set by twice as much as an equivalent increase in honey bee visitation. Visitation by wild insects and honey bees promoted fruit set independently, so pollination by managed honey bees supplemented, rather than substituted for, pollination by wild insects. Our results suggest that new practices for integrated management of both honey bees and diverse wild insect assemblages will enhance global crop yields.

Human persistence depends on many natural processes, termed ecosystem services, which are usually not accounted for in market valuations. The global degradation of such services can undermine the ability of agriculture to meet the demands of the growing, increasingly affluent, human population (1, 2). Pollination of crop flowers by wild insects is one such vulnerable ecosystem service (3), as the abundance and diversity of these insects are declining in many agricultural landscapes (4, 5). Globally, yields of insect-pollinated crops are

often managed for greater pollination through the addition of honey bees (*Apis mellifera* L.) as an agricultural input (Fig. 1) (6–8). Therefore, the potential impact of wild pollinator decline on crop yields is largely unknown. Nor is it known whether increasing application of honey bees (9) compensates for losses of wild pollinators, or even promotes these losses.

Fruit set, the proportion of a plant's flowers that develop into mature fruits or seeds, is a key component of crop yield (fig. S1). Wild insects may increase fruit set by contributing to polli-

nator abundance, species number (richness), equity in relative species abundance (evenness), or some combination of these factors. Increased pollinator abundance, and therefore the rate of visitation to crop flowers, should augment fruit set at a decelerating rate until additional individuals do not further increase fruit set (e.g., pollen saturation) or even decrease fruit set (e.g., pollen excess) (10–12). Richness of pollinator species should increase the mean, and reduce the variance, of fruit set (13) because of complementary pollination among species (14, 15), facilitation (16, 17), or “sampling effects” (18), among other mechanisms (19, 20). Pollinator evenness may enhance fruit set via complementarity, or diminish it if a dominant species (e.g., honey bee) is the most effective pollinator (21). To date, the few studies on the importance of pollinator richness for crop pollination have revealed mixed results (22), the effects of evenness on pollination services remain largely unknown, and the impact of wild insect loss on fruit set has not been evaluated globally for animal-pollinated crops.

We tested four predictions arising from the assumptions that wild insects effectively pollinate a broad range of crops, and that their role can be replaced by increasing the abundance of honey bees in agricultural fields: (i) For most crops, both wild insect and honey bee visitation enhance pollen deposition on stigmas of flowers; (ii) consequently, for most crops, wild insect and honey bee visitation both improve fruit set; (iii) visitation by wild insects promotes fruit set only when honey bees visit infrequently (i.e., there is a negative interaction effect between wild insect visitation and honey bee visitation); and (iv) pollinator assemblages with more species benefit fruit set only when honey bees visit infrequently (i.e., there is a negative interaction effect between richness and honey bee visitation).

To test these predictions, we collected data at 600 fields on all continents, except Antarctica, for 41 crop systems (Fig. 1). Crops included a

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wide array of animal-pollinated, annual and perennial fruit, seed, nut, and stimulant crops; predominantly wind-pollinated crops were not considered (fig. S2 and table S1). The sampled fields were subject to a diversity of agricultural practices, including extensive monocultures and small or diversified systems (fig. S2 and table S1), fields stocked with low to high density of honey bees (Fig. 1 and table S2), and fields with low to high abundance and diversity of wild insects (fig. S3 and table S2). For each field, we measured flower visitation per unit of time (hereafter “visitation”) for each insect species, from which we estimated species richness and evenness (23). We quantified pollen deposition for 14 systems as the number of pollen grains per stigma, and fruit set (fig. S1) for 32 systems as the percentage of flowers setting mature fruits or seeds. Spatial or temporal variation of pollen deposition and fruit set were measured as the coefficient of variation (CV) over sample points or days within each field (10). The multilevel data provided by fields within systems were analyzed with general linear mixed-effects models that included crop system as a random effect, and wild insect visitation, honey bee visitation, evenness, richness, and all their interactions as fixed effects. Best-fitting models were selected on the basis of the Akaike information criterion (AIC) (23).

In agreement with the first prediction, crops in fields with more flower visits received more pollen on stigmas, with an overall 74% stronger influence of visitation by honey bees than by wild insects (Fig. 2A and table S3). Honey bee visitation significantly increased pollen deposition (i.e., confidence intervals for individual regression coefficients, β_i , did not include zero) in 7 of 10 crop systems, and wild insects in 10

of 13 systems (fig. S4). Correspondingly, increased wild insect and honey bee visitation reduced variation in pollen deposition among samples (fig. S5).

Contrary to the second prediction, fruit set increased significantly with wild insect visitation in all crop systems, but with honey bee visitation in only 14% of the systems (Fig. 2B). In addition, fruit set increased twice as strongly with visitation by wild insects as with visitation by honey bees (Fig. 2A). These partial regression coefficients did not differ simply because of unequal abundance, nor because of disparate variation in visitation between wild insects and honey bees. In crop systems visited by both honey bees and wild insects, honey bees accounted for half of the visits to crop flowers [mean = 51%; 95% confidence interval (CI) = 40 to 62%], and among-field CVs for visitation by honey bees (mean = 73%; 95% CI = 57 to 88%) and by wild insects (mean = 79%; 95% CI = 62 to 96%) were equivalent. Furthermore, wild insect visitation had stronger effects than honey bee visitation, regardless of whether honey bees were managed or feral (fig. S6) and, comparing across systems, even where only wild insects or honey bees occurred (Fig. 2B). Wild insect visitation alone predicted fruit set better than did honey bee visitation alone ($\Delta_{\text{AIC}} = 16$; table S4, model F versus model M). Correspondingly, the CV of fruit set decreased with wild insect visitation but varied independently of honey bee visitation (fig. S5).

Pollinator visitation affected fruit set less strongly than did pollen deposition on stigmas (compare regression coefficients in Fig. 2A). This contrast likely arose from pollen excess, filtering of pollen tubes by post pollination processes, and/or seed abortion (11, 24), and so reflects pol-

lination quality, in part. Intriguingly, the difference in coefficients between pollen deposition and fruit set for honey bees greatly exceeded that for wild insects (Fig. 2A); this finding indicates that wild insects provide better-quality pollination, such as greater cross-pollination (14, 16, 17, 19). These results occurred regardless of which crop systems were selected (fig. S7), sample size (fig. S8), the relative frequency of honey bees in the pollinator assemblage (dominance) among systems, the pollinator dependence of crops, or whether the crop species were herbaceous or woody, or native or exotic (fig. S9). Poor-quality pollination could arise if foraging behavior on focal resources typical of honey bees (16, 17) causes pollen transfer between flowers of the same plant individual or the same cultivar within a field, thereby limiting cross-pollination and increasing the incidence of self-pollen interference and inbreeding depression (24). The smaller difference in coefficients between pollen deposition and fruit set for wild insects, and the stronger effect of wild insect visitation on fruit set, suggest that management to promote diverse wild insects has great potential to improve the global yield of animal-pollinated crops.

The third prediction was also not supported. Fruit set consistently increased with visitation by wild insects, even where honey bees visited frequently (i.e., no statistical interaction; Fig. 2, A and C). In particular, the best-fitting model (lowest AIC) for fruit set included additive effects of visitation by both wild insects and honey bees (table S4, model P), which suggests that managed honey bees supplement the pollination service of wild insects but cannot replace it. Overall, visitations by wild insects and honey bees were not correlated among fields (fig. S10), providing no evidence either for

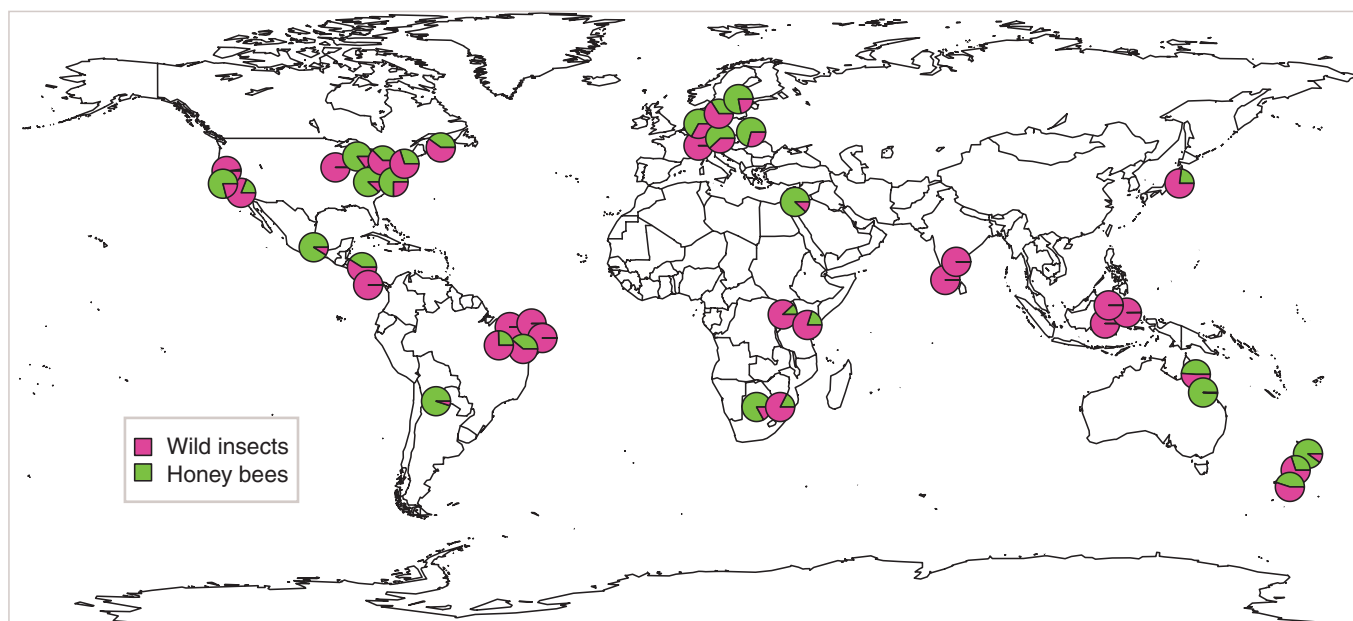


Fig. 1. Relative visitation by honey bees and wild insects to flowers of 41 crop systems on six continents. Honey bees occur as domesticated colonies in transportable hives worldwide, as a native species in Europe (rarely) and Africa, or as feral populations in all other continents except Antarctica.

competition for the resources obtained from crop flowers (pollen, nectar) or for density compensation (13) between wild insects and honey bees at the field scale. Even if honey bees displace wild insects (or vice versa) at the flower scale (16, 17), this is unlikely to scale up to the field, as indicated by our data, if mass-flowering crops provide floral resources in excess of what can be exploited by local pollinator populations. Therefore, insect pollinators appear not to be limited by crop floral resources, but crop yield was commonly pollen-limited, as crops set more fruit in fields with more visitation by pollinators (Fig. 2).

Contrary to the fourth prediction, fruit set increased with flower-visitor richness indepen-

dently of honey bee visitation (fig. S11). Correspondingly, the CVs of fruit set decreased with richness; in contrast, evenness did not affect the mean or CV of fruit set (figs. S12 and S13). Visitation by wild insects increased strongly with richness (Fig. 3) and improved model fit (lower AIC), even when richness was included in the model (table S4, model B versus model G). However, richness did not enhance model fit when added to a model with wild insect visitation (table S4, model F versus model G), which suggests that the effects of richness on fruit set reflect increased wild insect visitation (i.e., co-linear effects; fig. S13). Like wild insect visitation (fig. S10), richness did not correlate with honey bee visitation (table S5). Previous studies

have shown that agricultural intensification reduces both species richness of pollinator assemblages and wild insect visitation (4, 5, 13, 19). Our results for multiple crop systems further demonstrate that fields with fewer pollinator species experience less visitation by wild insects and reduced fruit set, independent of species evenness or honey bee visitation. Globally, wild insect visitation is an indicator of both species richness and pollination services, and its measurement can be standardized easily and inexpensively among observers in field samples (25). Large, active colonies of honey bees provide abundant pollinators that can be moved as needed, hence their appeal for pollination management in most animal-pollinated crops (6–8, 26). By

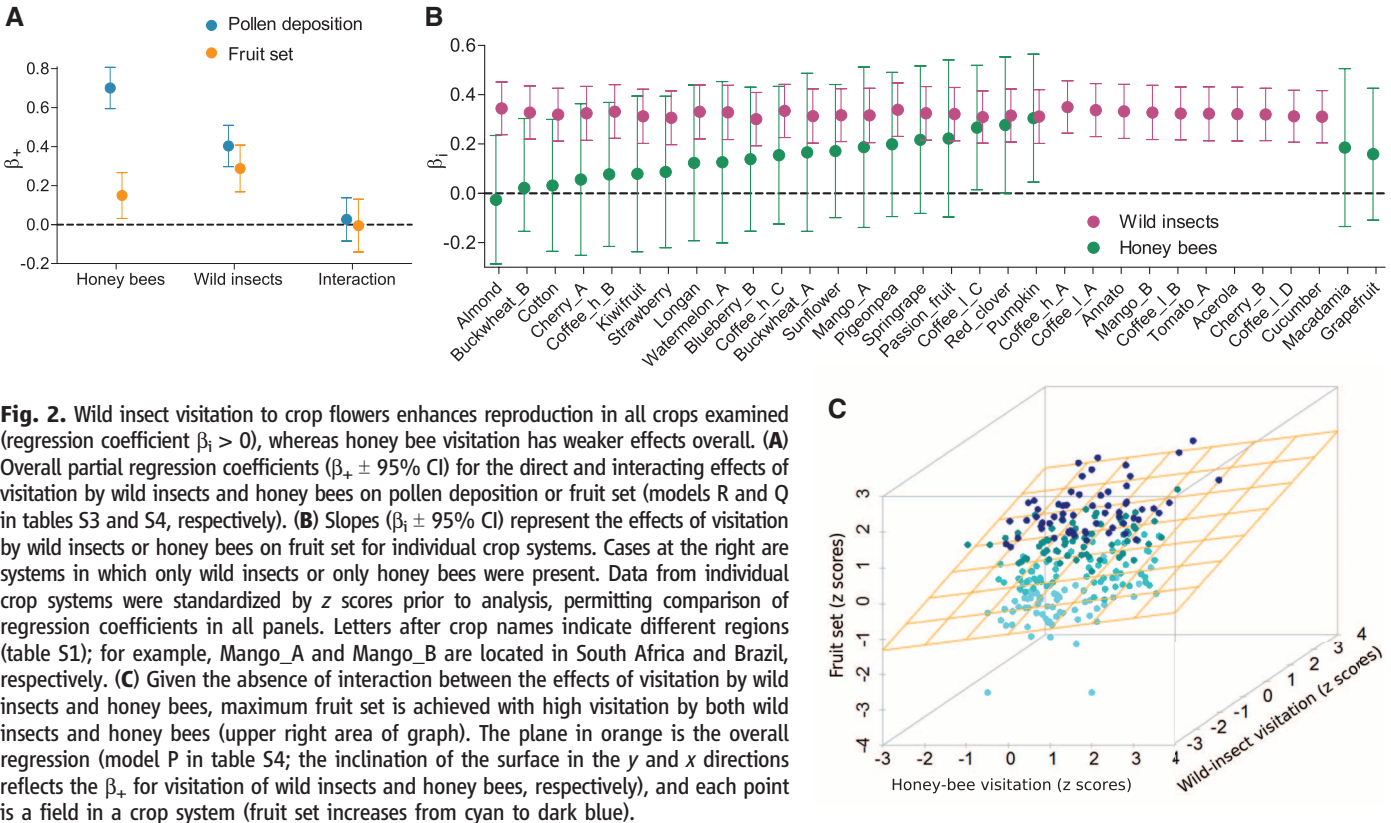


Fig. 2. Wild insect visitation to crop flowers enhances reproduction in all crops examined (regression coefficient $\beta_i > 0$), whereas honey bee visitation has weaker effects overall. **(A)** Overall partial regression coefficients ($\beta_+ \pm 95\% \text{ CI}$) for the direct and interacting effects of visitation by wild insects and honey bees on pollen deposition or fruit set (models R and Q in tables S3 and S4, respectively). **(B)** Slopes ($\beta_i \pm 95\% \text{ CI}$) represent the effects of visitation by wild insects or honey bees on fruit set for individual crop systems. Cases at the right are systems in which only wild insects or only honey bees were present. Data from individual crop systems were standardized by z scores prior to analysis, permitting comparison of regression coefficients in all panels. Letters after crop names indicate different regions (table S1); for example, Mango_A and Mango_B are located in South Africa and Brazil, respectively. **(C)** Given the absence of interaction between the effects of visitation by wild insects and honey bees, maximum fruit set is achieved with high visitation by both wild insects and honey bees (upper right area of graph). The plane in orange is the overall regression (model P in table S4; the inclination of the surface in the y and x directions reflects the β_+ for visitation of wild insects and honey bees, respectively), and each point is a field in a crop system (fruit set increases from cyan to dark blue).

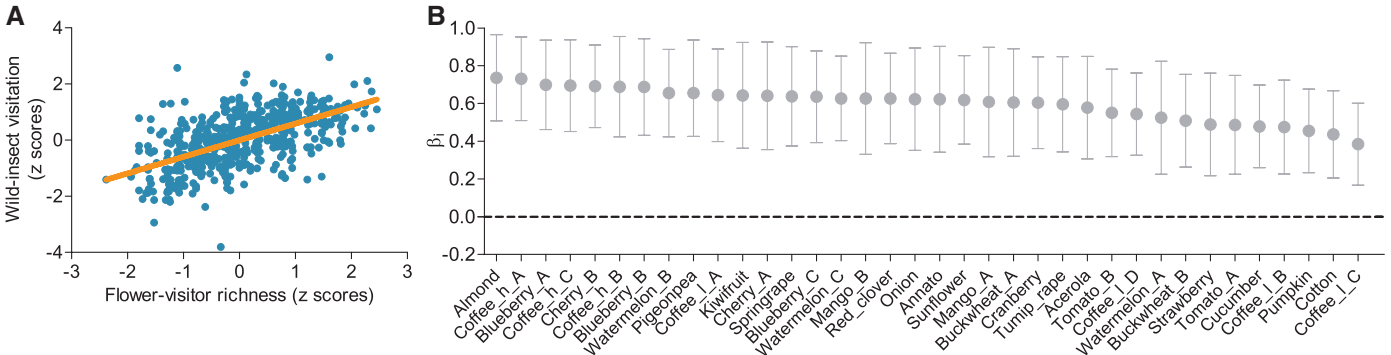


Fig. 3. Globally, rate of visitation to crop flowers by wild insects increases with flower-visitor richness. **(A)** The line is the overall regression, and each point is a field in a crop system. **(B)** Slopes ($\beta_i \pm 95\% \text{ CI}$) represent the effect of richness

on wild insect visitation for individual crop systems. Data from individual crop systems were standardized by z scores prior to analysis (after log-transformation for visitation), permitting direct comparison of regression coefficients.

comparison, methods for maintaining diverse wild insects for crop pollination are less developed, and research on such pollination services is more recent (3, 16, 17, 20, 26, 27) (table S1). Although honey bees are generally viewed as a substitute for wild pollinators (3, 6–8), our results show that they neither maximize pollination nor fully replace the contributions of diverse wild insect assemblages to fruit set for a broad range of crops and agricultural practices on all continents with farmland. These conclusions hold even for crops stocked routinely with high densities of honey bees for pollination, such as almond, blueberry, and watermelon (Fig. 2 and table S2). Dependence on a single species for crop pollination also carries the risks associated with predator, parasite, and pathogen development (4, 20, 28).

Our results support integrated management policies (29) that include pollination by wild insects as ecosystem service providers, along with managed species—such as honey bees, bumble bees (*Bombus* spp.), leafcutter bees (*Megachile* spp.), mason bees (*Osmia* spp.), and stingless bees (*Meliponini*)—as agricultural inputs, where they are not invasive species. Such policies should include conservation or restoration of natural or seminatural areas within croplands, promotion of land-use heterogeneity (patchiness), addition of diverse floral and nesting resources, and consideration of pollinator safety as it relates to pesticide application (3, 16, 17, 20, 27). Some of these recommendations entail financial and op-

portunity costs, but the benefits of implementing them include mitigation against soil erosion as well as improvements in pest control, nutrient cycling, and water-use efficiency (30). Without such changes, the ongoing loss of wild insects (4, 5) is destined to compromise agricultural yields worldwide.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1230200/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S13
Tables S1 to S5
References (31–79)
Database S1

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Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function

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Using historic data sets, we quantified the degree to which global change over 120 years disrupted plant-pollinator interactions in a temperate forest understory community in Illinois, USA. We found degradation of interaction network structure and function and extirpation of 50% of bee species. Network changes can be attributed to shifts in forb and bee phenologies resulting in temporal mismatches, nonrandom species extinctions, and loss of spatial co-occurrences between extant species in modified landscapes. Quantity and quality of pollination services have declined through time. The historic network showed flexibility in response to disturbance; however, our data suggest that networks will be less resilient to future changes.

Almost 90% of flowering plant species, including many important crop species (1), rely on animal pollinators (2). Plant-pollinator interaction networks may be particularly susceptible to anthropogenic changes, owing to their sensitivity to the phenology, behavior,

physiology, and relative abundances of multiple species (3). Alternatively, the overall structure of plant-pollinator networks might be robust to perturbations because of a high degree of nestedness and redundancy in interactions (4).

Several authors have speculated about how changes in biodiversity (5) and phenology (6–8) might translate into changes in the structure (9, 10) and stability (11) of complex interaction networks. However, there has been a lack of historical data on plant-pollinator networks and phenologies for both plants and insects in the same community.

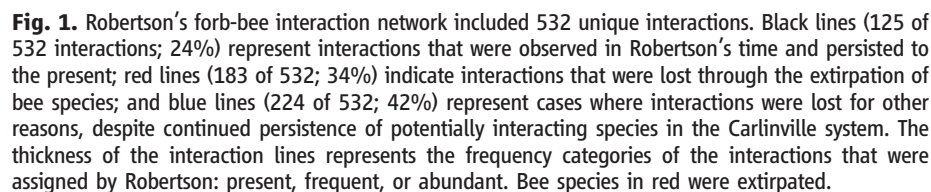
By using an extensive and unique data set, we were able to examine changes in plant-pollinator network structure and phenologies of forbs and bees across more than a century of anthropogenic change.

In the late 1800s, Charles Robertson meticulously collected and categorized insect visitors to plants, as well as plant and insect phenologies, in natural habitats near Carlinville, Illinois, USA (12–14). Over the next century, this region experienced severe habitat alteration, including conversion of most forests and prairies to agriculture, and moderate climatic warming of 2°C in winter and spring. In 2009 and 2010, we revisited the area studied by Robertson and re-collected data on the phenologies and structure of a subset of this network—26 spring-blooming forest understory forbs and their 109 pollinating bees (15). Hence, we could quantify changes in network structure, local bee diversity, and phenologies of forbs and bees. Further analyses and a null model determined the degree to which changes in network structure and bee diversity were attributed to species' traits, phenological mismatches, and land-use factors that spatially separate interacting species. To examine shifts in the quantity of pollinator services, we used a second historical data set from Carlinville collected in the early 1970s (16), examining the diversity and visitation rate of bees to the most important floral resource in this network (*Claytonia virginica*).

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Bee extirpations contributed substantially to the observed shifts in network structure. Of the 407 lost interactions, 45% (183) were lost because bee species were extirpated from the study region; all 26 forbs remained present. It is unlikely that the dramatic loss of bees observed in the contemporary data set resulted from differences in sampling effort between the historic and contemporary studies. Robertson observed the pollinators of each forb species for 1 to 2 years before moving on to other species. In our intensive resurvey over 2 years, we found less than half (54 of 109) of those bee species. Although Robertson's sampling effort in each season is unknown, we were able to extrapolate our data based on sampling effort (17) and found that our observations were close to the "true" richness (table S1). If Robertson's sampling was less intense on a per plant species basis than ours, then the bee extirpations are a conservative estimate. Furthermore, the loss of bees was nonrandom, such that bees that were specialists, parasites, cavity-nesters, and/or those that participated in weak historic interactions were more likely to be extirpated (table S2), congruent with other findings (18, 19). Specialists were lost more than generalists (even after correcting for potential observation bias), despite the fact that their host plants were still present (table S2 and fig. S1). This pattern may result from lower specialist abundances in Robertson's time (fig. S1) and/or their higher sensitivity to fluctuations in floral resources (20) and habitat loss (21). Parasitic species (mostly in the genus *Nomada*; family Apidae) were lost more than solitary or social bees, possibly because of the greater sensitivity of higher trophic levels to habitat loss and other perturbations (22). Additionally, cavity-nesting species (many in the Megachilidae family) (fig. S2) were lost disproportionately (table S2), potentially related to landscape management that reduces the availability of woody debris for their nests. Persisting bee species participated in stronger interactions historically [i.e., greater mean phenological



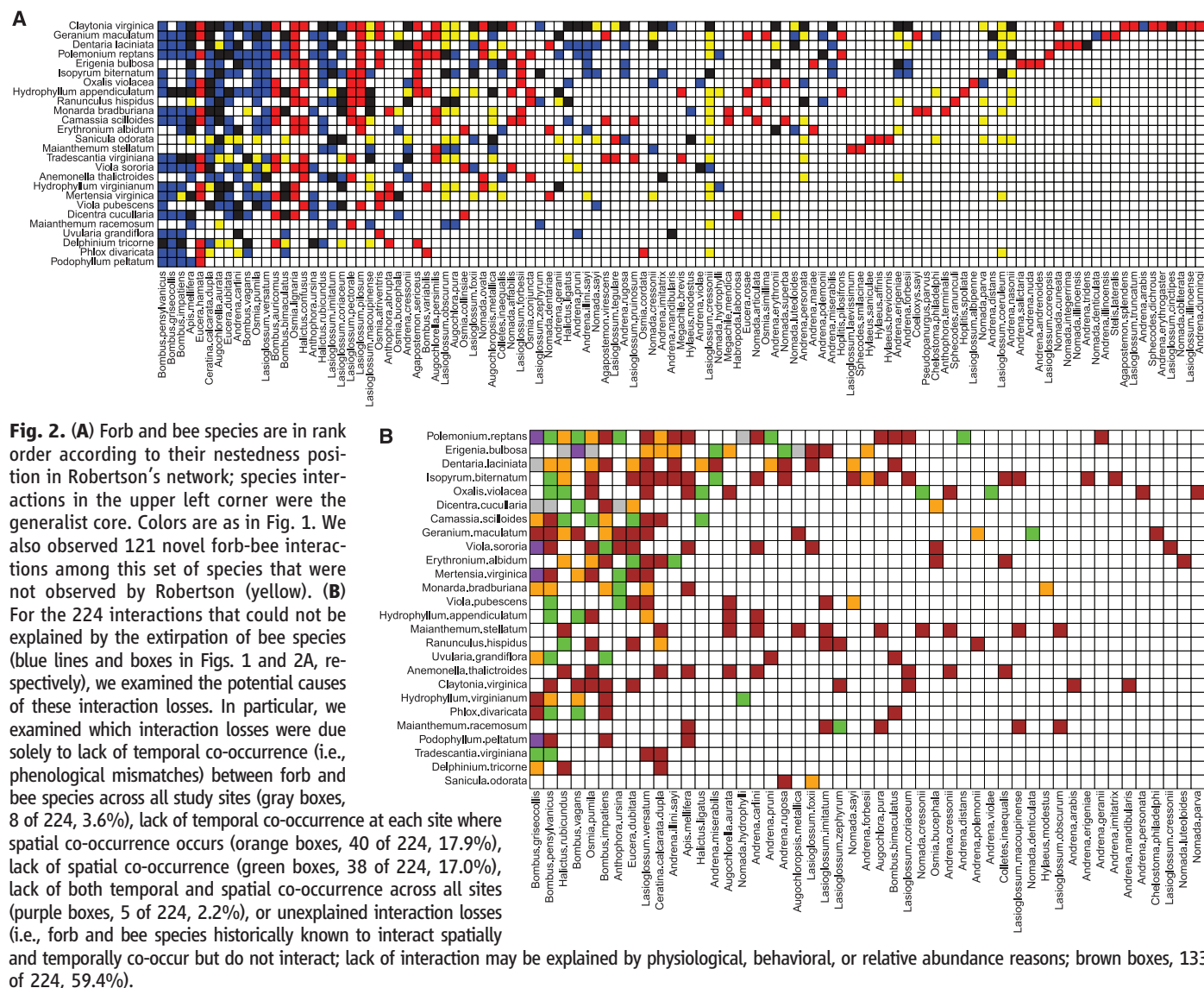
overlap (23) by over 4 days (table S2)]. Many of these factors are not significantly associated with extinction when information on phylogenetic relationships are incorporated into statistical analyses (table S2), possibly because traits tend to be clustered in a few clades and/or few contrasts are available in the taxonomic phylogeny (fig. S2).

Historic sampling occurred in a relatively continuous forest landscape, whereas our modern observations were constrained to remaining forest fragments within a matrix of agricultural, commercial, and residential lands. Of the 224 lost interactions not explained by the extirpation of bee species, 41% (91 of 224) were explained by either lack of spatial co-occurrence (38 of 91), lack of temporal co-occurrence (48 of 91, phenological mismatches), or both (5 of 91) (Fig. 2). The contemporary networks are vulnerable to future perturbations because remaining interactions often occur at only a single study site and across a very short temporal period (e.g., 73% occurred during <1 week).

Few studies have examined phenological changes in both forb and bee communities in the same location across a long period (8). Recent literature syntheses suggest that forb and bee communities should shift synchronously, because the phenologies of both are strongly influenced by temperature (9). Alternatively, it is possible that bees rely more on temperature for their development and activity (9), whereas forbs use a more diverse suite of cues (24, 25), resulting in phenological mismatches. We found evidence for the latter. Peak forb bloom was 9.5 days earlier ($t_{25} = 3.91$, $P = 0.0007$), and peak bee activity was 11 days earlier ($t_{29} = 5.92$, $P < 0.0001$) in 2009 to 2010; both results are on par with previous observations from other systems [plants (6, 7); pollinators (8, 26, 27)]. However, phenologies of bee species active earliest in the spring shifted the most ($F_{1,29} = 5.89$, $P = 0.022$, $r = 0.42$) (fig. S3), whereas there were no differences in phenological shifts among forb species ($F_{1,25} = 0.0001$, $P = 0.99$, $r = 0.0024$) (fig. S3). Moreover, bloom periods were 8 days

shorter ($t_{25} = 3.18$, $P = 0.0042$) and flight periods were 22.5 days shorter ($t_{29} = 4.67$, $P < 0.0001$), likely from physiological responses and/or reduced population sizes with truncated phenological variance (28). These results compounded to weaken interaction strengths [i.e., phenological overlap (23)] through time ($t_{657} = 2.55$, $P = 0.011$).

We devised a null model approach to disentangle the likely contributions of these phenological shifts versus other possible mechanisms in bee extirpation and interaction losses. The null model uses real data about historic interactions and phenology and observed phenological shifts in extant forbs and bees. Model scenarios examine a range of possible shifts in bee phenology (because the phenology of extirpated bees is not known) and circumstances under which bees and forbs forge novel interactions. Null-expected bee extirpations and loss of interactions due solely to phenological shifts ranged from 17 to 55% and 14 to 44% of those observed, respectively (figs. S4 and S5). Both the



null-expected (fig. S6) ($F_{1,98} = 27.35$, $P < 0.0001$) and observed (above) results showed higher extinction for more specialized bees. Other non-random bee extirpations are not explained by phenological changes and may result from the major shifts in the landscape that occurred over the past 120 years.

Interaction gains, losses, and rewiring contributed substantially to the observed shifts in network structure. We observed large changes in the diet breadth of species that persisted. Changes in the species' relative abundances, behavioral shifts, and evolutionary responses [mutualism abandonment (29)] may all have contributed to these shifts. Studies examining plant-pollinator interactions across several years also report substantial rewiring resulting from fluctuations in species' relative abundances across years, showing that such changes in networks can occur even in shorter periods (30). However, we constructed networks by summing across years for the historic and contemporary sampling periods (albeit with few years within a sampling period), and some of the species in our network experienced population declines across decades (31). Historically, Apinae (primarily bumblebees) had significantly wider diet breadths than other bee groups ($F_{8,100} = 4.34$, $P = 0.0002$) but have experienced the greatest loss of interactions ($F_{7,46} = 5.45$, $P < 0.0001$). This was due in part to recent population declines of some species (31), such as *Bombus pensylvanicus*, the most connected bee in Robertson's data set; we only observed one individual in 447 hours of sampling, highlighting its severely reduced role in network structure. Interestingly, remaining and novel interactions were redistributed across bee species, not just historic generalists (figs. S7 and S8). As a result of the combined influence of bee extirpations, interaction losses, and diet breadth shifts (interaction rewiring), the overall structure of the forb-bee interaction network became less nested than it was historically (fig. S9), indicative of increased vulnerability of pollination services to future perturbations (4).

Changes in network structure and species abundance might be expected to alter both the diversity of visitors to forbs and the service pollinators are providing (quantity and quality of pollen delivered). In particular, bee extirpations may result in lowered interspecific competition among remaining species, decreasing fidelity (32). Alternatively, if community-wide declines in floral resources resulted in heightened competition among bees, fidelity may increase. To examine these patterns more explicitly, we focused on bee visitors to *Claytonia virginica*, one of the most important floral resources during early spring, both in terms of abundance and diversity of pollinators. We used a second historical data set on the pollinators of this species in 1971 (16) from the same field sites as those visited in 2009 to 2010. First, we found that the richness of bee species visiting *C. virginica* did not change between Robertson's studies and 1971 but de-

clined by over half in the past 40 years (Fig. 3 and table S3), which appeared to be largely driven by changes in forested habitat area (change in forested habitat during the past 40 years was significantly related to change in bee species richness visiting *C. virginica*; $F_{1,11} = 6.62$, $P = 0.028$, $r = 0.63$, $\Delta\text{bee richness} = 0.073 + 0.000093 \times \Delta\text{forest area}$). Second, we found that rates of bee visitation to *C. virginica* were more than four times as high in the early 1970s as in the contemporary data (0.59 and 0.14 bees per minute, respectively; $t_{11} = 3.76$, $P = 0.0031$). Third, *C. virginica* bee community composition was nested across sampling sites in 1971 (i.e., poor sites housed

subsets of species that were found at better sites; $P = 0.03$), but they were not significantly nested in 2010 ($P = 0.67$) (fig. S10), suggesting a loss of redundancy in bee species that is characteristic of more intact communities. Finally, we quantified the proportion of *C. virginica* pollen grains on the bodies of representative specimens of six *Andrena* species that were captured during visits to *C. virginica* during the same three time periods and found that bee pollinators have almost three times lower fidelity now than 120 years ago (Fig. 4) ($F_{2,483} = 166.65$, $P < 0.0001$). Thus, each of these metrics showed that pollination service on *C. virginica* consistently declined.

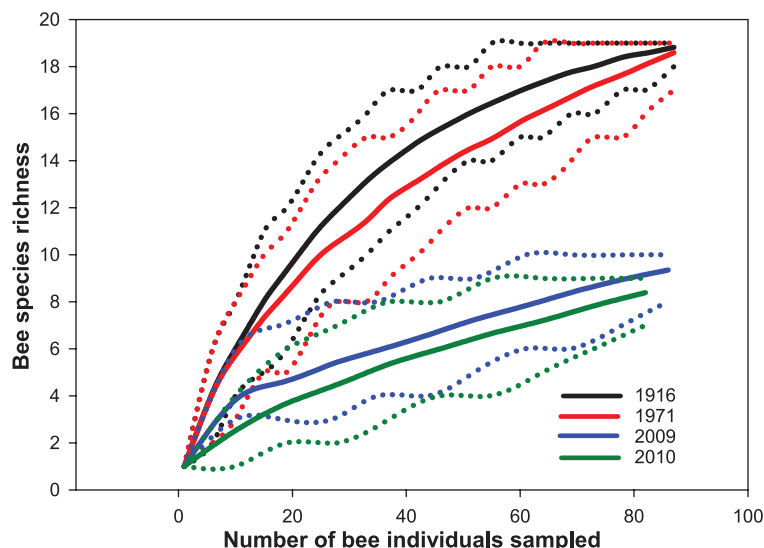


Fig. 3. The rarefied richness (solid lines) and 95% confidence intervals (dotted lines) of bee species richness visiting *Claytonia virginica* was more than twice as high in both 1916 and 1971 compared with 2009 and 2010.

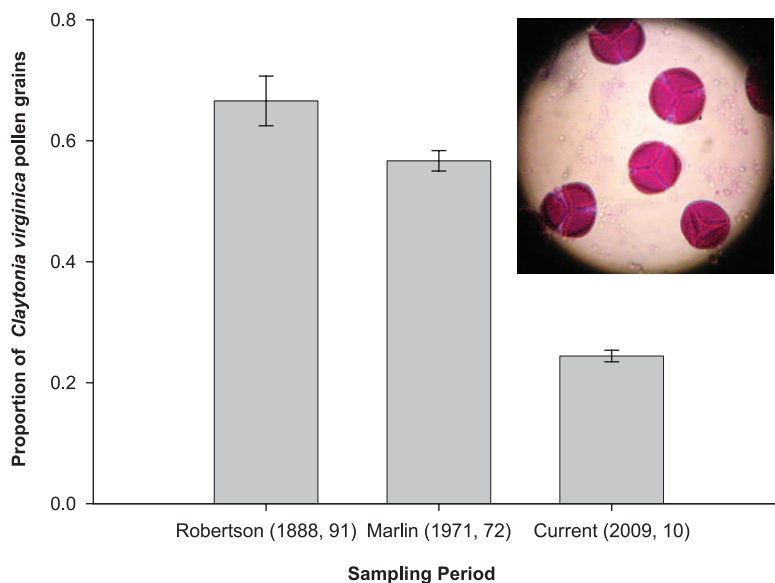


Fig. 4. Across six bee species, the proportion of *Claytonia virginica* pollen grains on the bodies of bee individuals captured visiting open flowers of that forb species declined over time, suggesting decreased fidelity and probability of successful pollination. Least-squared means are reported $\pm$ SE. Inset is a photo of *C. virginica* pollen grains.

We have found major changes in a plant-pollinator network over the past 120 years. This is partly explained by the nonrandom extirpation of bee species that are expected to be the most vulnerable to land-use and climate change, such as rare and specialized species, species occupying higher trophic levels, and cavity-nesting species. We found large changes in phenology of both forbs and pollinators and the potential for interaction mismatches, and these phenological changes can explain some of the species and interaction losses observed in this system. Our more optimistic finding was that plant-pollinator interaction networks were quite flexible in the face of strong phenological change and bee species extirpations, with many extant species gaining interactions through time. However, the redundancy in network structure has been reduced, interaction strengths have weakened, and the quantity and quality of pollinator service has declined through time. Further interaction mismatches and reductions in population sizes are likely to have substantial negative consequences for this crucial ecosystem service.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S10

Tables S1 to S3

References (33–37)

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Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest

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Boreal forest soils function as a terrestrial net sink in the global carbon cycle. The prevailing dogma has focused on aboveground plant litter as a principal source of soil organic matter. Using ¹⁴C bomb-carbon modeling, we show that 50 to 70% of stored carbon in a chronosequence of boreal forested islands derives from roots and root-associated microorganisms. Fungal biomarkers indicate impaired degradation and preservation of fungal residues in late successional forests. Furthermore, 454 pyrosequencing of molecular barcodes, in conjunction with stable isotope analyses, highlights root-associated fungi as important regulators of ecosystem carbon dynamics. Our results suggest an alternative mechanism for the accumulation of organic matter in boreal forests during succession in the long-term absence of disturbance.

Globally, the boreal forest biome covers 11% of the land surface (1) and contains 16% of the carbon (C) stock sequestered in soils (2). Aboveground plant litter quality and decomposition rates have been proposed as the fundamental determinants of long-term soil organic matter accumulation (3–6). However, a large proportion of photosynthetically fixed C is directed belowground to roots and associated microorganisms (7, 8), potentially affecting C sequestration either positively or negatively (9–12). A better mechanistic understanding of how the belowground allocation of C affects

long-term sequestration rates is crucial for predictions of how the currently large C stock in boreal forest soils may respond to altered forest management practices, climate change, elevated CO₂ levels, and other environmental shifts.

Here we present evidence from a fire-driven boreal forest chronosequence that enables the study of soil C sequestration over time scales of centuries to millennia. The system consists of forested islands in two adjacent lakes, Lake Hornavan and Lake Uddjaure (65°55' to 66°09'N; 17°43' to 17°55'E), in northern Sweden. The islands in these lakes were formed after the most recent

glaciation and have since been subjected to similar extrinsic factors. Larger islands, however, burn more frequently because they have a larger area to intercept lightning strikes (6, 13); several large islands have burned in the past century, whereas some small islands have not burned in the past 5000 years. It has previously been shown that as the time since fire increases, soil and total ecosystem C accumulates unabated and linearly (6, 14), leading to humus layers that can exceed 1 m in depth on the smallest islands. This has been attributed to a decline in the quality of aboveground litter inputs and impaired litter decomposition as the chronosequence proceeds (6, 14, 15). We studied organic soil profiles on 30 islands representing three size classes with increasing belowground C stocks (14): 10 large islands (>1.0 ha; on average, 6.2 kg of C m⁻² accumulated belowground; mean time since fire 585 years), 10 medium islands (0.1 to 1.0 ha, 11.2 kg of C m⁻², 2180 years), and 10 small islands (<0.1 ha, 22.5 kg of C m⁻², 3250 years).

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We explored C dynamics across the chronosequence by analyzing bomb ^{14}C (16) to determine the age since fixation of soil C, and then fitted a mathematical model to measurements of C mass and age distribution across vertical organic matter profiles for six representative islands; three large and three small (Fig. 1) (17). The model assumes two sources of C inputs: (i) a series of consecutively deposited cohorts of aboveground plant litter with negligible vertical mixing, and (ii) belowground inputs through root transport and rhizosphere processes. The dynamics of both C sources were estimated by a Bayesian parameterization of the model. The observed distribution of C mass and age was adequately predicted only when root C import was accounted for (Table 1 and figs. S1 and S2). The parameterized model estimates that the proportion of root-derived C accumulated over the past 100 years is larger on small islands (70%) than on large islands (47%), and the larger total C sequestration on small islands during this period can be explained entirely by root-derived inputs (Fig. 1). Differences in organic matter accumulation between islands were primarily determined by processes at the interface between the fragmented litter (F) and humus (H) layers, which corresponded to the zone of highest root density (Fig. 2B) and where the aboveground litter was 10 to 60 years old. The model was run for 100 years, covering almost the entire humus profile of the large islands, but on small islands a major proportion of C is stored in deeper horizons that are older than this. However, the model indicates that below 20 cm depth, root-derived C inputs are low and the C remaining from the horizons above decomposes slowly, as is also supported by ^{14}C depletion in the deeper layers of small islands (Table 1). Thus, root-mediated C input to the upper part of the profile represents a major contribution to the long-term buildup of humus, especially in late successional ecosystems.

Fungi play central roles in boreal forest ecosystems, both as decomposers of organic matter and as root-associated mediators of belowground C transport and respiration. We profiled the relative abundance of major functional groups of fungi through the depth profile of each island by DNA barcoding based on 454 pyro sequencing of the ITS2 region of ribosomal RNA genes (17, 18). These analyses suggest that fungal communities in the uppermost litter layers were dominated by free-living saprotrophs, whereas mycorrhizal and other root-associated fungi dominated at greater depth (Fig. 2A). Thus, root-associated fungi dominate the part of the soil profile where the model indicates the largest difference in C sequestration between the island size classes. At this depth, free-living saprotrophs (mainly molds and yeasts) make a much reduced contribution, suggesting a correspondingly greater role of root-associated fungi in the regulation of organic matter dynamics.

The increase in root-derived C sequestration as the chronosequence proceeds is matched by a

shift in the balance between the production and decomposition of fungal mycelium in the F-H transition zone of the soil profile. We measured the fungal-specific cell membrane lipid ergosterol as a marker for fungal biomass throughout each soil profile. Even though standing fungal biomass, as indicated by total (free plus bound) ergosterol (Fig. 3, A and B) and ITS copy numbers (table S1), was roughly similar on all islands, free ergosterol (characteristic of newly formed mycelia) (19–21) was about 20 times more abundant on large than on small islands, indicating a larger proportion of freshly produced mycelium and thus greater mycelial production. In contrast, bound ergosterol (the proportion of which increases during mycelial senescence) (19, 20) was more abundant on smaller islands, indicating older mycelium with slower biomass turnover.

Furthermore, the fungal cell-wall polysaccharide chitin (Fig. 3C) peaked in the F layer and declined in lower horizons of large islands, but remained at high concentrations at greater depths on the small islands. Chitin persists longer than ergosterol in fungal tissues after death (21), and the high level of chitin on small islands suggests retarded decomposition of fungal cell wall residues. Thus, in spite of supposedly greater mycelial production on the large islands, less mycelial necromass accumulated there than on small islands, suggesting that the large production was counterbalanced by faster decomposition of mycelial remains. Correspondingly, the ^{14}C model indicated faster decomposition of root-derived C on large islands, despite inputs being conservatively constrained to be equal across all islands. Taken together, our results point to impaired

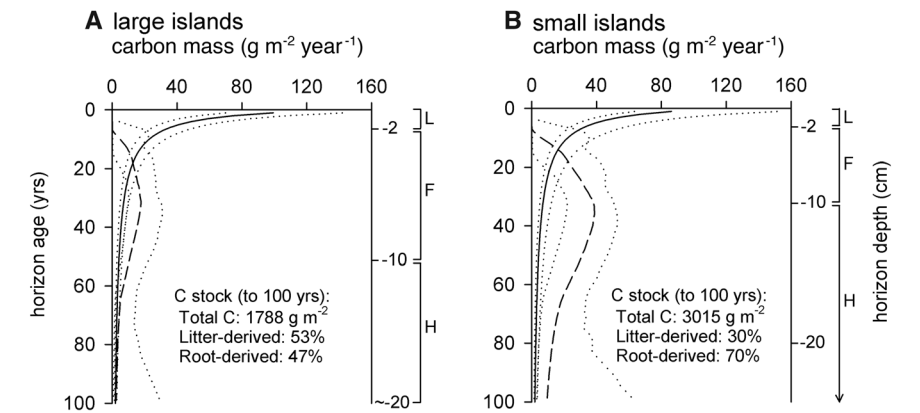


Fig. 1. Carbon dynamics in vertically stratified organic horizons of forested islands. Model estimates of C from aboveground litter (solid lines) and C introduced belowground via root transport (broken lines) are shown. C mass was modeled to a horizon age of 100 years, based on C mass and ^{14}C measurements in profiles from three large (A) and three small (B) islands. Dotted lines show the 95% central credibility intervals around posterior means. The posterior probability that the root-derived fraction is larger on small islands than on large islands is 0.97. Approximate depths are indicated for transitions between the main categories of horizons sampled; L, litter; F, fragmented litter; H, humus.

Table 1. C mass, ^{14}C abundance, and estimated C mean age of sampled organic layers on large and small islands. Means $\pm$ SE, $n = 3$ ($n = 2$ for the deepest layer in both size classes; both values are given).

Layer	Large islands			Small islands		
	C mass (g m^{-2})	$\Delta^{14}\text{C}$ (‰)	C age (years)	C mass (g m^{-2})	$\Delta^{14}\text{C}$ (‰)	C age (years)
Litter, on surface	103 $\pm$ 3	70 $\pm$ 3	7 $\pm$ 0	95 $\pm$ 7	62 $\pm$ 1	6 $\pm$ 0
Litter, 0 to 2 cm	185 $\pm$ 17	81 $\pm$ 17	9 $\pm$ 3	180 $\pm$ 11	84 $\pm$ 11	9 $\pm$ 2
Fragmented litter, 2 to 7 cm	454 $\pm$ 91	118 $\pm$ 12	15 $\pm$ 2	539 $\pm$ 63	101 $\pm$ 14	12 $\pm$ 2
Fragmented litter, 7 to 10 cm	493 $\pm$ 76	165 $\pm$ 19	20–39*	494 $\pm$ 77	136 $\pm$ 7	18 $\pm$ 1
Humus, 10 to 16 cm	1170 $\pm$ 90	150 $\pm$ 31	42–51*	1150 $\pm$ 165	191 $\pm$ 14	23–33*
Humus, 16 to 20 cm	1620 $\pm$ 230	21 $\pm$ 16	53 $\pm$ 1	910 $\pm$ 105	195 $\pm$ 17	34–50*
Humus, 20 to 40 cm	3410, 1846	9.4, -14.3	53, 57	6440 $\pm$ 815	14 $\pm$ 15	59 $\pm$ 7
Humus, 40 to 60 cm				7660 $\pm$ 700	-99 $\pm$ 14	780 $\pm$ 120
Humus, 60 to 80 cm				7380 $\pm$ 1760	-194 $\pm$ 9	1670 $\pm$ 90
Humus, 80 to 100 cm				5620, 3650	-285, -267	2628, 2432

*The mean age is within the given interval in samples that include the 1960s peak in bomb ^{14}C .

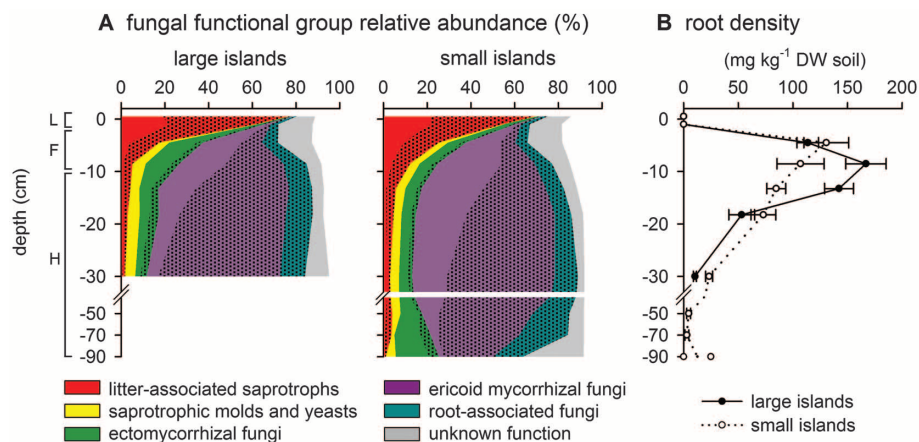


Fig. 2. Depth profiles of the relative abundance of fungal functional groups (percent of amplified ITS sequences) (A) and root (1 to 5 mm in diameter) density (B) on large and small forested islands. The profile corresponds to the organic layers L, F, and H. Functional groups comprise identified species with known function (unshaded) and species putatively assigned to a function (shaded) (17). The data set contains 650,000 sequence reads, and the globally most abundant 583 clusters are analyzed, covering 82 to 95% of the reads in individual sample types. The total ITS copy number was not affected by island size but decreased with depth (with 3×10^9 , 4×10^8 , and 2×10^7 copies g^{-1} of organic matter in L, F, and H layers, respectively) (table S1). The abundances of different functional groups should be compared with caution because of possible differences in ITS copy numbers per unit of biomass. All values are based on means of $n = 10$ islands (except that $n = 2, 5$, and 7 for the lowest horizons) (17).

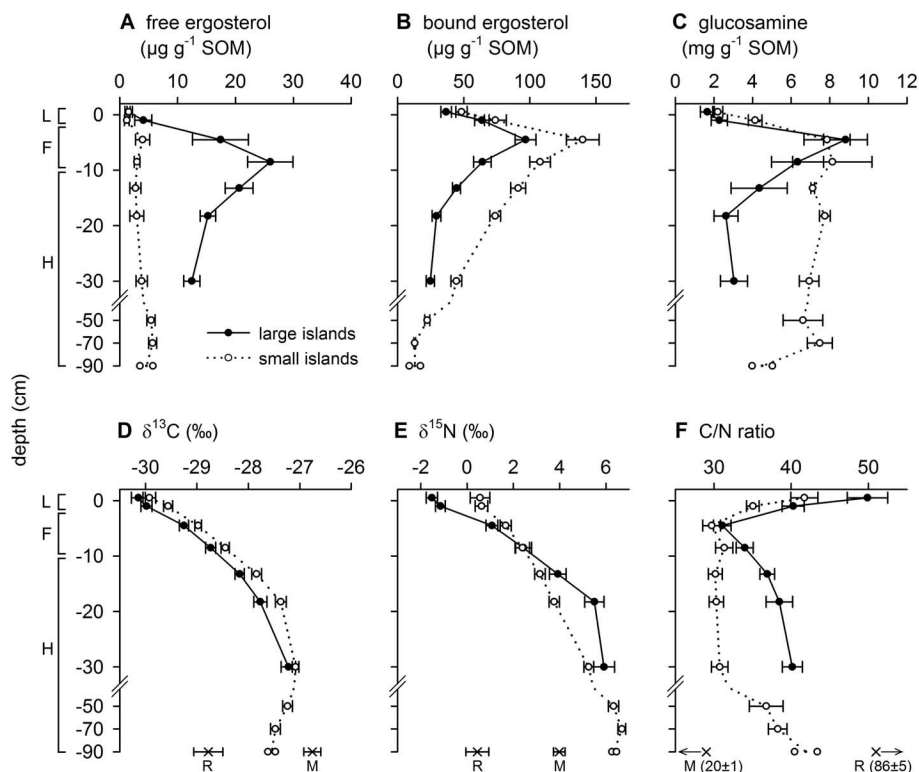


Fig. 3. Depth profiles of fungal biochemical markers (A to C), $\delta^{13}\text{C}$ (D), $\delta^{15}\text{N}$ (E), and the C/N ratio (F) in organic soil profiles of large (solid lines) and small (broken lines) forested islands. All data are means $\pm$ SE, $n = 10$ (except that $n = 3$ for glucosamine and $n = 2, 5$, and 7 for the lowest horizons) (17). Medium-sized islands are not shown but are included in the statistical analyses presented in table S1. In the lower panel, levels measured in roots (R) 1 to 5 mm in diameter and mycorrhizal mycelium (M) sampled at 10 cm depth are given for reference.

decomposition of fungal residues as an important regulator of C accumulation as the chrono-sequence proceeds.

The often observed increases in ^{13}C and ^{15}N abundances with soil depth have been interpreted as evidence of increasing contributions of enriched microbial components to residual soil organic matter (22–24). In our system, where the dominant plant species form ecto- or ericoid mycorrhizal associations, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in the uppermost organic layers were similar to those of leaves (25), whereas signatures in humus layers were closer to those of rhizosphere mycelium (Fig. 3, D and E, and table S1) and mycorrhizal fungal sporocarps (26). Plant C allocated belowground is relatively enriched in ^{13}C , and this enrichment is further accentuated during C transfer to mycorrhizal fungi (27). However, historic changes in atmospheric $\delta^{13}\text{C}$ (28) may also contribute to the depth gradient (22, 24). Mycorrhizal fungi also have higher $\delta^{15}\text{N}$ signatures than their host plants, because they supply N to their hosts that is ^{15}N -depleted relative to that retained in their own mycelium (29). Thus, the incorporation of isotopically enriched root and fungal remains is likely to be an important mechanism behind the increasing stable isotope signatures with soil depth in this system. This is consistent with the observation that isotopic signatures remain relatively constant in the initial litter decomposition phase and only increase when root-associated fungi dominate C and N dynamics (Figs. 2 and 3) (30, 31).

Previous studies in this (6, 14) and other (3, 4) systems have pointed to the input and quality of aboveground litter as important regulators of C and N sequestration during long-term ecosystem development and succession. Our results show that aboveground plant litter dynamics on its own cannot explain the increasing rate of organic matter accumulation with time since wildfire, and that the dynamics of roots and associated fungi is an important additional factor explaining C accumulation in boreal forests. Although we observe less C accumulation on large islands, it is reasonable to assume that C allocation to roots and associated mycelium is greatest on those islands, especially given their higher root densities (Fig. 2B), free ergosterol levels (Fig. 3A), and net primary productivity (6). This apparent contradiction corroborates recent results (32, 33) showing that increased C input to roots in response to CO_2 enrichment accelerates the turnover of soil organic matter, counteracting C accumulation and enhancing N cycling through the microbial pools. In our system, a similar stimulation of N recycling by large C inputs is supported by the steeper ^{15}N gradient (31) and higher C:N-ratio in the humus of large islands (Fig. 3, E and F) (30). In contrast, the less steep ^{15}N gradient and lower C:N-ratio on smaller islands suggest impaired mycorrhizal N mobilization (31) and accumulation of N in biochemically stabilized fungal remains, consistent with the high levels of bound ergosterol and

chitin on those islands. The consequential reduced N availability to plants leads to progressive nutrient limitation and compositional changes in the vegetation with increasing time since a major disturbance (14, 34). Changes in plant productivity and community composition may, in turn, influence total belowground C allocation and distribution to fungal associates. Together, these feedbacks result in continuing C and N accumulation in the humus layer and decreasing plant production, and this process is only reset by major disturbances, such as wildfire.

Our results elucidate the mechanisms underpinning C sequestration in boreal forests and highlight the importance of root-associated fungi for ecosystem C balance and, ultimately, the global C cycle. We challenge the previous dogma that humus accumulation is regulated primarily by saprotrophic decomposition of aboveground litter, and envisage an alternative process in which organic layers grow from below through the continuous addition of recently fixed C to the organic matter profile in the form of remains from roots and associated mycelium. Environmental changes, such as N fertilization and deposition, forest management, and elevated atmospheric CO₂ concentrations, are therefore likely to greatly affect soil C sequestration through their alteration of rhizosphere processes. These processes are not well described in current models of ecosystem and global C dynamics, and their more explicit inclusion is likely to improve both the mechanistic realism and future predictive power of models.

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Supplementary Materials

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The Biological Underpinnings of Namib Desert Fairy Circles

Norbert Juergens

The sand termite *Pсамmotermes allocerus* generates local ecosystems, so-called fairy circles, through removal of short-lived vegetation that appears after rain, leaving circular barren patches. Because of rapid percolation and lack of evapotranspiration, water is retained within the circles. This process results in the formation of rings of perennial vegetation that facilitate termite survival and locally increase biodiversity. This termite-generated ecosystem persists through prolonged droughts lasting many decades.

Fairy circles (FCs) are large, conspicuous, circular patches devoid of vegetation in the center but with perennial grasses at the margin. These patches occur in large numbers in the desert margin grasslands of southern Africa (Fig. 1, A and B). Early observers considered poisonous plants, ants, or termites as causal factors; however, most of these early hypotheses were systematically tested and rejected (1, 2). It has also been proposed that an unknown semi-volatile substance in the soil might be respon-

sible for the absence of grass within the FCs (2, 3). In fact, a wide range of volatile organic compounds are found in FCs (4). Measurements of carbon monoxide and hydrocarbons in the soil led to the proposal of a geochemical origin of FCs (5). Carnivorous ants (6) and “self-organizing vegetation dynamics” (7) have also been considered as causes for FCs. Despite the many hypotheses, the origin and the ecosystem function of FCs are still a much-debated mystery. I used a long-term data set describing the environmental and biogeographical characteristics and dynamics of FCs to identify the most likely cause of these unique formations. Additionally, I analyze the function of FCs in terms of

water management, biodiversity, and adaptation to arid conditions.

FCs occur along a narrow belt at the eastern margin of the Namib Desert, running from mid-Angola to northwestern South Africa. The area of distribution is closely associated with the isohyet of 100-mm mean annual precipitation (MAP) (Fig. 1B). The disjunct occurrence of FCs is caused by their pronounced restriction to sandy soils.

High soil humidity within FCs has been observed previously (1, 2). To confirm and quantify this potentially adaptive function, I measured volumetric soil water content ($\text{m}^3/\text{m}^3 \times 100$) from 2006 to 2012 within and around FCs. At sites with a MAP of 100 mm, more than 53 mm of water were stored in the upper 100 cm of soil, even during the driest time of the year (table S1). At a depth >40 cm, a soil humidity of more than 5% volumetric water content was recorded over 4 years.

Higher temporal resolution of water flux was gained by automatic measurements recorded every hour within the bare patch and the grass matrix at 10-, 30-, 60-, and 90-cm depths using FDR sensors. During the observation period of 4 years, the humidity at 60-cm depth within the FC was either at or well above 5% volumetric water content (Fig. 2A). In the typical sand texture of FC soils with dominant grain sizes around

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180 μm and pore sizes around 50 μm , 5% volumetric water content causes more than 98% relative air humidity in the pore system.

I hypothesize that the FC, while not losing water by transpiration because of the absence of plants in its center, accumulates rain water as a result of the rapidly draining large pore size of sand. The rapid percolation to a deeper soil layer reduces evaporation loss. Simultaneous measurements taken under the matrix vegeta-

tion showed much lower water contents (Fig. 2B). The amount and the longevity of the water body underneath the FC allow the formation of a belt of perennial grasses at its margin. Their roots extend only 20 to 30 cm into the bare patch. The majority of FCs possess such a perennial belt (PB), and they are essential for the ecosystem functioning of the FCs. Here, I test the hypothesis of a biogenic origin by interpreting the area of distribution of FCs as a clearly defined

environmental envelope (80 to 120 mm MAP, deep sandy soils) of an organismic taxon causing intraspecific competition for space (2).

Data collected over 40 field trips were used to systematically assess which organisms are associated with FCs. At each site (Fig. 1B), at least 30, and in some cases more than 100, FCs were investigated above and below ground.

Species distribution maps show that only a few organisms are associated with FC hotspots across their entire distribution (table S2). Among termites, only the sand termite (*Psammotermes allocerus*) was found at all FC hotspots, whereas *Hodotermes mossambicus* is largely restricted to the summer rainfall climate and *Microhodotermes viator* is limited to the winter rainfall climate. *Baicaliotermes hainsii* only occurs south of the southern Central Namib. *P. allocerus* is widely distributed over southern Africa and thus exceeds the FC distribution. Three ant species—*Messor denticornis*, *Anoplolepis steingroeveri*, and *Tetramorium* sp.—were found in several FC hotspots, but none of them in all.

If direct presence of organisms in or next to all single FCs (table S2) is scrutinized, only *P. allocerus* was found in high frequencies (80 to 100%). The characteristic “sheetings” (thin layers of cemented sand built over the foraged plant material) of *P. allocerus* (fig. S2, A, C, and D) were found at 80 to 100% of the FCs and throughout all life stages of the FCs. In addition, in 80 to 100% of FCs, *P. allocerus* nests (Fig. 3B and fig. S12) and underground tunnel-like galleries with a characteristic black organic wall covering (tapetum) (figs. S9C and S12, C and E) were found a few centimeters to decimeters underneath the bare patch, the PB, and the matrix area. The frequency of these observations was halved during the wet season.

Although these associations suggest a causal role for *P. allocerus*, it is possible that they may instead merely reflect the colonization of FCs by the termites. However, sand termites were found even in the initial state of new FCs, that is, before the water accumulation has begun and the perennial grass belt has developed. Careful assessment of 24 newly formed FCs at Giribesvlakte in Namibia in March 2012 revealed the presence of *P. allocerus* in all of them. In these youngest FCs, the dying grass plants were damaged only at the roots, associated with underground galleries of *P. allocerus* (fig. S9C). No other organism has been observed foraging on the grass of young FCs.

During the further life history of FCs, *P. allocerus* is directly involved in keeping the bare patch of FCs free of grass. The related presence and activity of *P. allocerus* can be best assessed at night and in the morning, when the workers clean the underground burrows and create characteristic small soil dumps (Fig. 3A and fig. S10). Within a random stratified sample of 83 FCs at Giribesvlakte, a negative correlation was found between the density of the soil dumps at the bare patch and the number of surviving grass

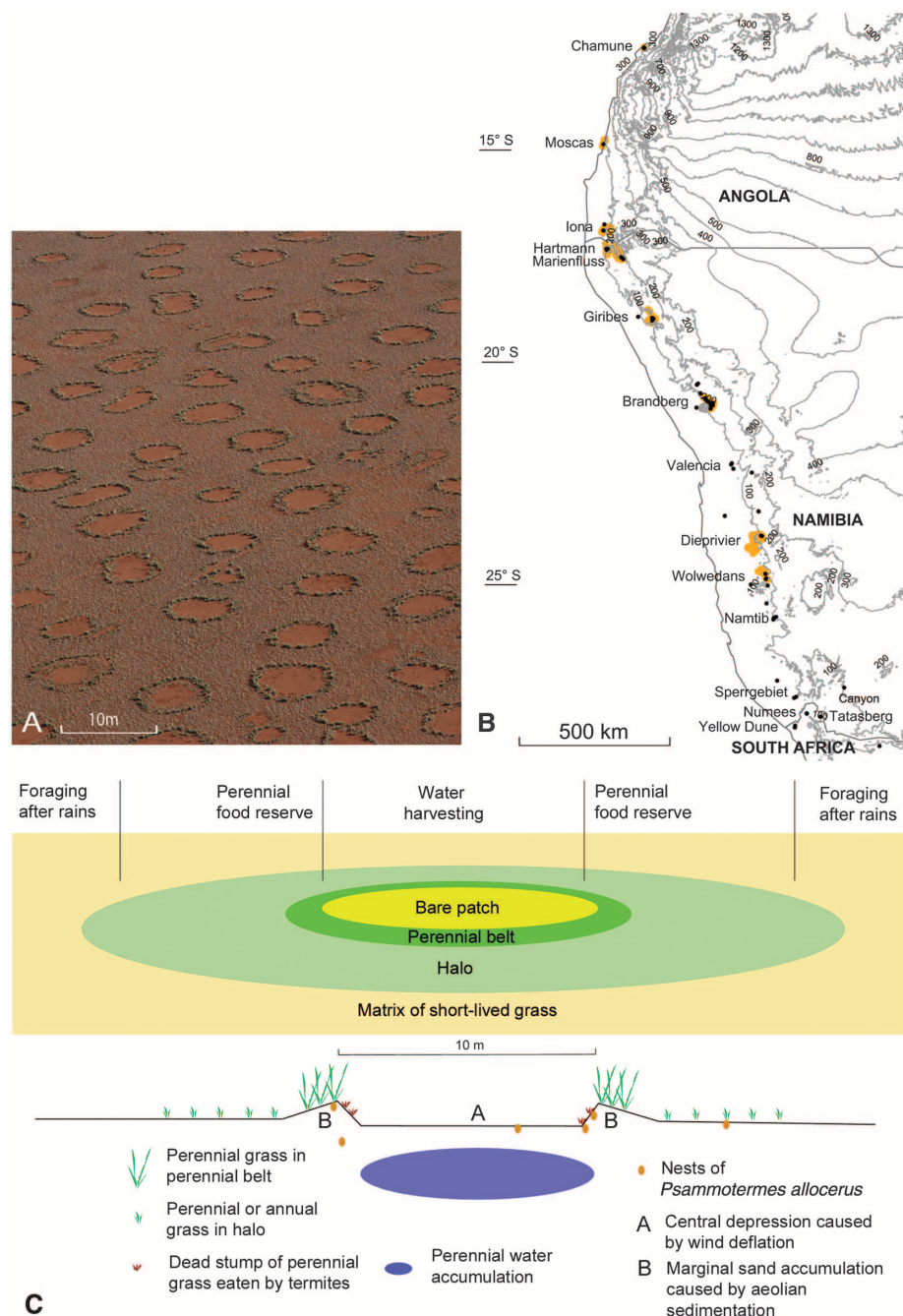


Fig. 1. (A) Spatial pattern of FCs. (B) Geographical distribution of FCs (black dots) and hotspots of FC occurrences at wider landscape scale (yellow clusters). Note the proximity of the occurrences to the 100-mm isohyet. (C) Schematic sketch showing the structural elements within a FC. The PB is formed by a ring of much larger perennial plants located around the bare patch. The grass outside the FC dies and disappears during drought. The PB remains the only surviving vegetation biomass during drought periods.

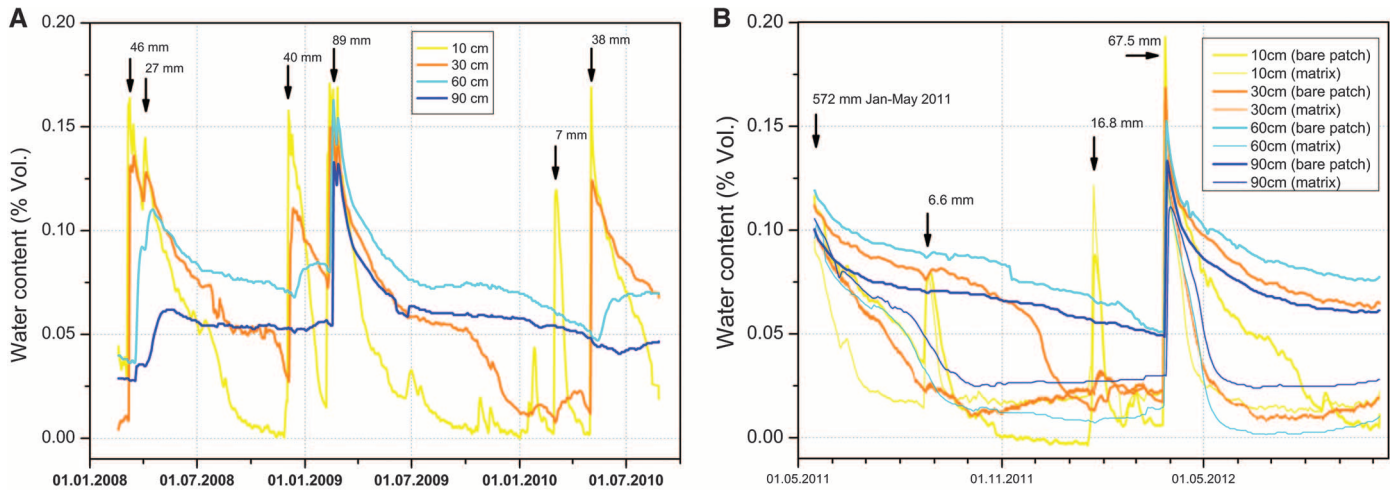


Fig. 2. (A) Rainfall events and volumetric soil water content (volume percent, $\text{m}^3/\text{m}^3 \times 100$) at different depths underneath a FC, measured hourly from early 2008 until mid-2010. **(B)** Same measurements as in (A) comparing the bare patch (FC, solid lines) and the matrix (MT, thin lines) measured from 15 May 2011 until 6 October 2012.

plants (Fig. 3C). These correlations suggest that the burrowing activities of *P. allocerus* within the bare patch do not only serve in taking up water (8); their foraging on the roots of freshly germinated grasses kills them and keeps the bare patch free of vegetation. Furthermore, *P. allocerus* is involved in widening the diameter of the circle. During most of the FCs' adulthood, the termites steadily feed on a few (often neighboring) perennial grass plants at the inner margin of the PB (figs. S2A, S11D, and S12B), thereby slowly widening the diameter of the FC. Out of a total of 160 FCs examined at Giribesvlakte in March 2011, 96% showed remains of dead grass tussocks at the margin of the bare patch. At 53% of these FCs, some of the grass tussocks—on average, 24.9 (minimum of 1, maximum 80)—were still covered with *P. allocerus* sheetings.

The main ecosystem function of FCs is related to securing two important perennial long-term resources. First, the removal of all water-transpiring plants allows the accumulation of water underneath the FC after rain events (water trap). I hypothesize that the generation of a perennial water supply facilitates the survival of termites in a hostile desert. Whereas the annual rainfall evenly distributed in space allows ephemeral or annual plant growth, the removal of plants allows perennial growth of plants in the PB. I argue that this generation of perennial plant biomass is the second facilitator of survival of termites, even in extreme drought years. The manner in which the termites create and manage the perennial grass population within an otherwise ephemeral desert environment supports the hypothesis of active ecosystem “engineering.” The formation of the PB is a consequence of the water accumulation and the unidirectional suppression of competition, both caused by the termites.

FCs strongly enhance biodiversity by attracting many organisms. Evidence of this was es-

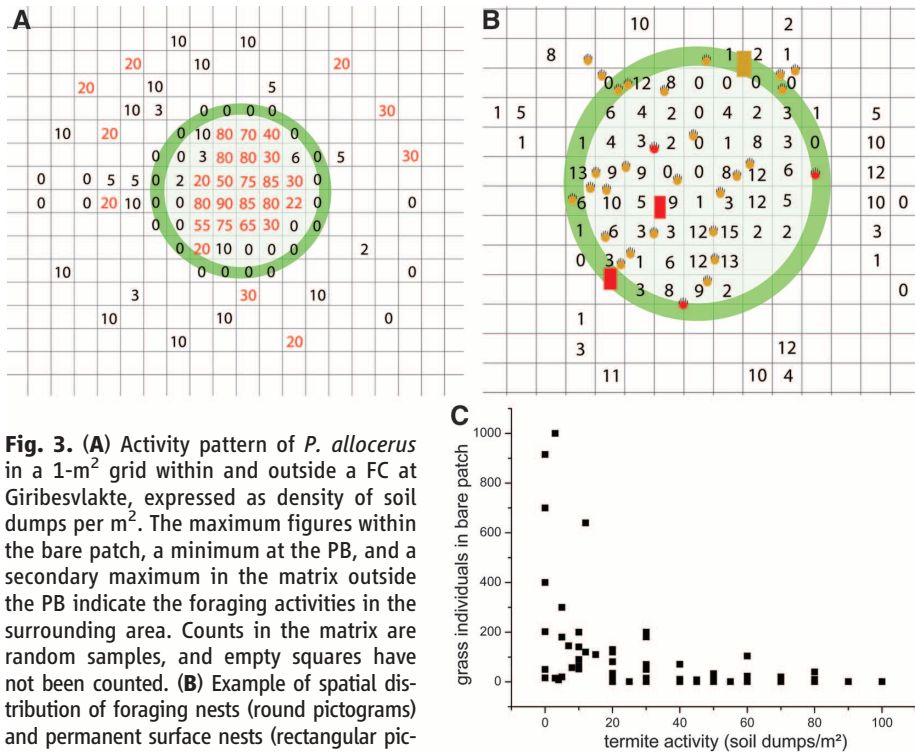


Fig. 3. (A) Activity pattern of *P. allocerus* in a 1-m² grid within and outside a FC at Giribesvlakte, expressed as density of soil dumps per m². The maximum figures within the bare patch, a minimum at the PB, and a secondary maximum in the matrix outside the PB indicate the foraging activities in the surrounding area. Counts in the matrix are random samples, and empty squares have not been counted. **(B)** Example of spatial distribution of foraging nests (round pictograms) and permanent surface nests (rectangular pictograms) (nests with living termites colored red; abandoned nests, ochre). The green ring marks the PB. **(C)** Assessment of 83 FCs with regard to the number of living grass plants (individuals) found in the bare patch, plotted against the termite activity, and measured as average number of soil dumps per m² within the bare patch.

tablished by comparing lists of taxa observed in and near FCs with lists established in nearby grasslands without FCs. A number of ants, bees, wasps, small mammals, and plants are found more often in and near FCs. Often the mainly granivorous ant *M. denticornis* establishes itself in the center of the bare patch and forages along linear foraging trails in the wider surroundings of the FC. Plant species, for example, the Cucurbitaceae *Citrullus lanatus* with its

large water-storing fruits and even *Acacia erioloba* trees, establish themselves within or next to FCs in the reticulate dunes at the eastern margin of the Namib dune field. Furthermore, the population of *P. allocerus* termites itself forms an attractive resource, which is used by geckos (*Palmatogeco rangei*), aardvarks (*Orycteropus afer*), bat-eared foxes (*Otocyon megalotis*), black-backed jackals (*Canis mesomelas*), golden moles (*Eremitalpa granti*), and spiders (e.g., *Seothyra*)

as well as by omnivorous ants like *Anoplolepis steingroeveri* (6) and *Tetramorium* sp., which have been regularly observed attacking *P. allocerus* workers. In summary, FCs, like oases in the desert, increase biodiversity (quantified as the number of species) by one to two orders of magnitude (table S3).

FCs can be regarded as an outstanding example of allogenic ecosystem engineering resulting in unique landscapes with increased biodiversity, driven by key resources such as permanently available water, perennial plant biomass, and perennial termite biomass. The termites match the beaver (9) with regard to intensity of environmental change, but they surpass it with regard to the spatial dimension of their impact. *P. allocerus* turns wide desert regions of predominantly ephemeral life into landscapes dominated by species-

rich perennial grassland (Fig. 1A and figs. S1C, S6, and S7), supporting uninterrupted perennial life even during dry seasons and drought years.

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Supplementary Materials

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(R)-2-Hydroxyglutarate Is Sufficient to Promote Leukemogenesis and Its Effects Are Reversible

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Mutations in *IDH1* and *IDH2*, the genes coding for isocitrate dehydrogenases 1 and 2, are common in several human cancers, including leukemias, and result in overproduction of the (R)-enantiomer of 2-hydroxyglutarate [(R)-2HG]. Elucidation of the role of *IDH* mutations and (R)-2HG in leukemogenesis has been hampered by a lack of appropriate cell-based models. Here, we show that a canonical *IDH1* mutant, *IDH1* R132H, promotes cytokine independence and blocks differentiation in hematopoietic cells. These effects can be recapitulated by (R)-2HG, but not (S)-2HG, despite the fact that (S)-2HG more potently inhibits enzymes, such as the 5'-methylcytosine hydroxylase TET2, that have previously been linked to the pathogenesis of *IDH* mutant tumors. We provide evidence that this paradox relates to the ability of (S)-2HG, but not (R)-2HG, to inhibit the EglN prolyl hydroxylases. Additionally, we show that transformation by (R)-2HG is reversible.

Acute myeloid leukemia (AML) is caused by somatic genetic mutations that deregulate hematopoietic cell proliferation and differentiation. In many cases of AML, the responsible genetic abnormalities are chromosomal translocations involving key transcription factors, epigenetic regulators, and mediators of cell signaling. However, no translocations are detected in 40% of cases of AML (1). In such cases of normal cytogenetic AML (NC-AML), the pathogenic driver mutations are largely unknown.

Recent genomic sequencing efforts, however, have identified a number of recurrent mutations in NC-AML that might contribute to leukemogenesis, including mutations in isocitrate dehydrogenases 1 and 2 (*IDH1* and *IDH2*) (2). *IDH1* and *IDH2* are key metabolic enzymes that convert isocitrate to α -ketoglutarate [also called 2-oxoglutarate (2OG)], which is an essential cofactor for 2OG-dependent dioxygenases. These enzymes are linked to diverse cellular processes such as adaptation to hypoxia, histone demethylation, and DNA modification (3). Cancer-associated *IDH* mutants convert 2OG to the (R)-enantiomer of 2-hydroxyglutarate [(R)-2HG] (4, 5). Both the (R)- and (S)-enantiomers of 2HG are structurally similar to 2OG and can inhibit many 2OG-dependent enzymes in vitro and in vivo (6–8). In the case of the EglN (Egg-laying defective Nine) prolyl hydroxylases that down-regulate the HIF (hypoxia-inducible factor) transcription factor, however, (R)-2HG potentiates EglN activity, whereas (S)-2HG inhibits this same activity (8). Therefore, mutant *IDH* is widely

believed to transform cells by modulating the behavior of specific 2OG-dependent enzymes. Nonetheless, *IDH* mutations induce a number of metabolic abnormalities in addition to (R)-2HG accumulation (9), and it has not yet been formally proven that (R)-2HG is sufficient to transform cells. Deciphering the pathogenic roles of mutant *IDH* and (R)-2HG in leukemia has been particularly problematic due to the lack of *IDH* mutant leukemic cell lines and the lack of robust cell-based assays with which to monitor hematopoietic transformation by mutant *IDH* and (R)-2HG.

To address this latter deficiency, we stably infected the TF-1 human erythroleukemia cell line with lentiviral vectors encoding hemagglutinin-tagged versions of wild-type (WT) *IDH1*, a tumor-derived mutant (*IDH1* R132H, where R132H denotes Arg¹³²→His¹³²), or an *IDH1* R132H variant in which three conserved aspartic acid residues within the *IDH1* catalytic domain were replaced with asparagines (R132H/3DN; D, Asp; N, Asn) (Fig. 1A). This leukemic line is unusual insofar as it is cytokine-dependent [granulocyte-macrophage colony-stimulating factor (GM-CSF)] and retains the ability to differentiate in response to erythropoietin (EPO) (10).

As expected, 2HG levels were dramatically increased in cells producing *IDH1* R132H, but not in cells producing WT *IDH1* or the catalytically inactive R132H/3DN variant (Fig. 1B). In multiple independent experiments, TF-1 cells expressing *IDH1* R132H became cytokine-independent 12 to 16 days (four passages) after infection (Fig. 1C). In contrast, parental TF-1 cells spontaneously became cytokine-independent, but with a much longer and more variable latency. Furthermore, after 10 passages in culture, *IDH1* R132H-expressing TF-1 cells, in contrast to the control cells, no longer differentiated in response to EPO (Fig. 1, D and E, and fig. S1). Thus, expression of mutant *IDH* in TF-1 cells promotes two hallmarks of leukemic transformation: growth factor independence and impaired differentiation. Of note, *IDH1* R132H impaired the fitness of TF-1

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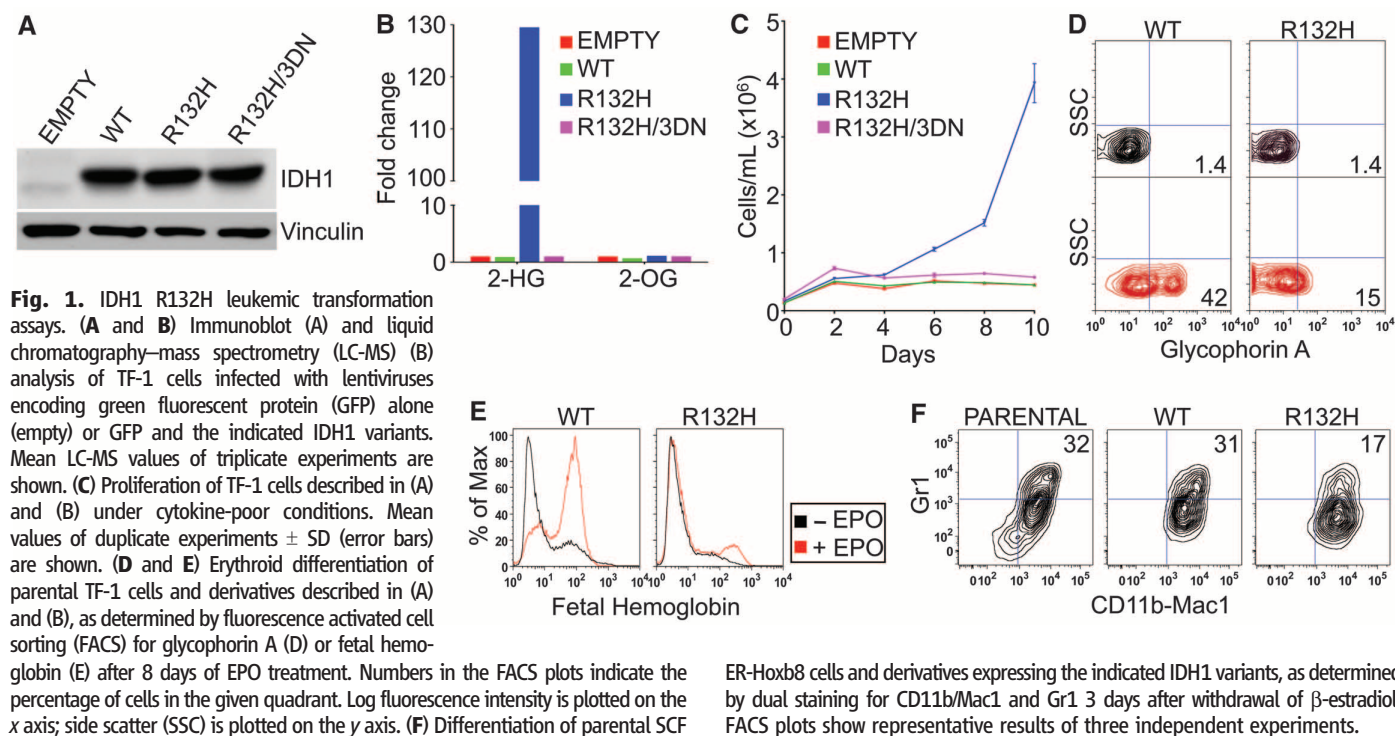
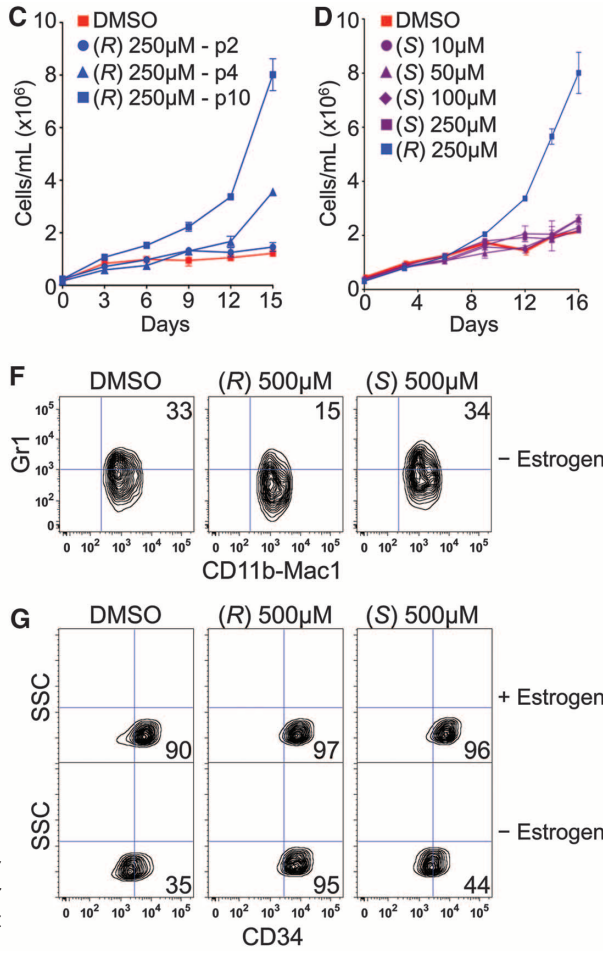


Fig. 1. IDH1 R132H leukemic transformation assays. **(A and B)** Immunoblot **(A)** and liquid chromatography–mass spectrometry (LC-MS) **(B)** analysis of TF-1 cells infected with lentiviruses encoding green fluorescent protein (GFP) alone (empty) or GFP and the indicated IDH1 variants. Mean LC-MS values of triplicate experiments are shown. **(C)** Proliferation of TF-1 cells described in **(A)** and **(B)** under cytokine-poor conditions. Mean values of duplicate experiments $\pm$ SD (error bars) are shown. **(D and E)** Erythroid differentiation of parental TF-1 cells and derivatives described in **(A)** and **(B)**, as determined by fluorescence activated cell sorting (FACS) for glycophorin A **(D)** or fetal hemoglobin **(E)** after 8 days of EPO treatment. Numbers in the FACS plots indicate the percentage of cells in the given quadrant. Log fluorescence intensity is plotted on the x axis; side scatter (SSC) is plotted on the y axis. **(F)** Differentiation of parental SCF

Fig. 2. (R)-2HG is sufficient to promote leukemogenesis. **(A)** LC-MS analysis of TF-1 cells after treatment for 3 hours with DMSO (–) or 250 μ M of the indicated esterified (TFMB) 2HG. Mean values of triplicate experiments are shown. **(B to D)** Proliferation of TF-1 cells under cytokine-poor conditions in the presence of the indicated amounts of TFMB–(R)-2HG **(B and C)** or TFMB–(S)-2HG **(D)**. Cells in **(B)** and **(D)** were passaged 10 times before GM-CSF withdrawal. Cells treated with 250 μ M TFMB–(R)-2HG are also included in **(D)** for comparison. Mean values of duplicate experiments $\pm$ SD (error bars) are shown. **(E)** Differentiation of TF-1 cells, as determined by glycophorin A FACS, after 8 days of EPO treatment following pretreatment for 10 passages with DMSO or 500 μ M TFMB-2HG (R or S enantiomer). Representative results of three independent experiments are shown. **(F and G)** Differentiation of SCF ER-Hoxb8 cells, as determined by dual staining for CD11b/Mac1 and Gr1 **(F)** or staining for CD34 **(G)**, 3 days after withdrawal of β -estradiol following pretreatment with DMSO or 500 μ M TFMB-2HG (R or S) for 20 passages. Representative results of two independent experiments are shown.



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cells grown in the presence of GM-CSF, despite conferring a proliferative advantage to TF-1 cells in the absence of GM-CSF (Fig. 1C and fig. S2). Consistent with this observation, IDH1 R132H expression was attenuated in TF-1 cells after repeated passaging in the presence of GM-CSF (fig. S3).

To confirm that the inhibitory effect of mutant IDH on hematopoietic differentiation is not restricted to TF-1 cells, we stably infected stem cell factor (SCF) estrogen receptor (ER)-Hoxb8 cells, which are granulocyte-macrophage progenitor cells derived from primary murine bone marrow cells immortalized with a conditional oncogene (11), with viruses encoding WT IDH1 or IDH1 R132H (fig. S4). In the presence of estrogen, these cells express a functional ER-Hoxb8 fusion protein that promotes their survival and proliferation. Upon estrogen withdrawal, the cells differentiate and up-regulate expression of the monocytic markers CD11b/Mac1 and Gr1. Expression of IDH1 R132H in SCF ER-Hoxb8 cells, however, blunted their differentiation in response to estrogen withdrawal (Fig. 1F).

To investigate whether the effects of IDH1 R132H on TF-1 cells are mediated by (R)-2HG, we treated TF-1 cells with vehicle [dimethyl sulfoxide (DMSO)] or cell membrane-permeable [trifluoromethyl benzyl (TFMB)-esterified] versions of either (R)-2HG or (S)-2HG. Measurement of intracellular 2HG levels in treated TF-1 cells confirmed that the esterified 2HG enantiomers were equally cell-permeable (Fig. 2A). TF-1 cells

passed in the presence of TFMB-(R)-2HG became growth factor-independent and no longer differentiated in response to EPO (Fig. 2, B and E). Promotion of growth factor independence and loss of EPO responsiveness by TFMB-(R)-2HG were dose-dependent (Fig. 2B and fig. S5) and passage-dependent (Fig. 2C and fig. S5). In contrast, TFMB-(S)-2HG did not promote cytokine independence or block differentiation at any concentration or time point tested (Fig. 2, D and E). Likewise, treatment of SCF ER-Hoxb8 cells with TFMB-(R)-2HG, but not TFMB-(S)-2HG, impaired their differentiation in response to estrogen withdrawal, as determined by decreased expression of Gr1 and persistent expression of the stem cell markers CD34 and c-kit (Fig. 2, F and G, and fig. S6). Thus, (R)-2HG, but not (S)-2HG, promotes leukemic transformation. Of note, absolute quantification of (R)-2HG levels in TF-1 cells transformed by expression of IDH1 R132H or by treatment with TFMB-(R)-2HG confirmed that the intracellular 2HG concentrations achieved in both settings approximate those found in IDH mutant neoplasms in patients (low millimolar values) (fig. S7) (12, 13). The accumulation of 2HG to these high levels in cells treated with 250 to 500 μ M TFMB-(R)-2HG suggests that the free 2HG liberated from the ester by intracellular esterases disappears more slowly than TFMB-(R)-2HG enters the cells.

To explore how (R)-2HG promotes leukemic transformation, we next infected parental

TF-1 cells with a pool of lentiviral short hairpin RNA (shRNA) vectors targeting all the known 2OG-dependent dioxygenases (four to eight shRNAs per enzyme) (table S1), cultured the cells in the absence of GM-CSF, and monitored the abundance of the individual shRNA vectors by next-generation DNA sequencing. We reasoned that the enzymes targeted by shRNAs that were substantially enriched over time (because, for example, those shRNAs confer cytokine independence) would contribute to transformation by (R)-2HG if these enzymes were also inhibited by (R)-2HG at concentrations observed in leukemic cells. One of the top-scoring enzymes from this screen was TET2 (Ten Eleven Translocation 2), which is mutationally inactivated in a subset of AML and has recently been suggested to be a pathogenic target of mutant IDH (Fig. 3A) (14, 15). In contrast, the TET2 paralog TET1 did not score in our screen (Fig. 3A).

We confirmed that TET2 knockdown, but not TET1 knockdown, recapitulated the ability of IDH1 R132H and (R)-2HG to promote cytokine independence and block differentiation of TF-1 cells, suggesting that TET2 inhibition contributes to transformation by mutant IDH (Fig. 3, B and C, and fig. S8). However, this finding created a paradox because (S)-2HG is a more potent inhibitor of TET2 than is (R)-2HG (6, 8), and yet TFMB-(R)-2HG, but not TFMB-(S)-2HG, promoted TF-1 cell transformation (Fig. 2). Moreover, TFMB-(S)-2HG, but not TFMB-(R)-2HG,

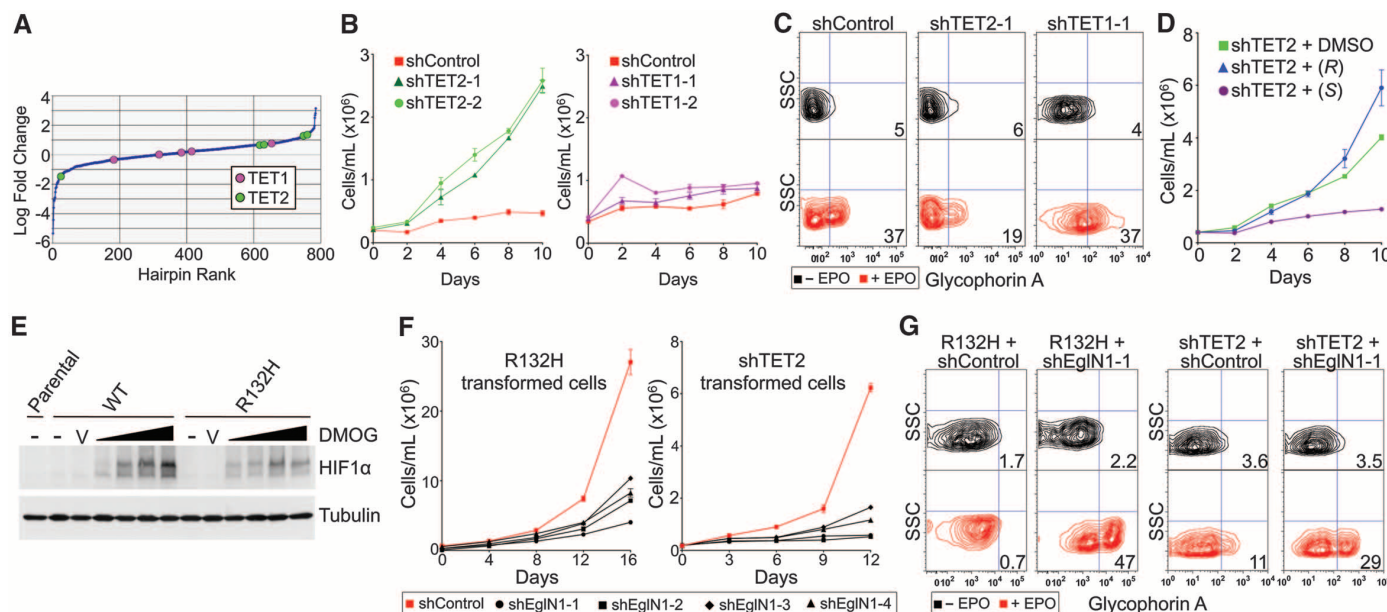


Fig. 3. Opposing roles of TET2 and EglN1 in TF-1 cell transformation. (A) Relative enrichment and depletion of shRNAs targeting TET2 or TET1 in TF-1 cells infected with a pool of ~800 lentiviral shRNA vectors targeting 2OG-dependent dioxygenases (four to eight shRNAs per gene) and then grown under cytokine-poor conditions for 10 days. Mean values of triplicate experiments are shown. (B and C) Proliferation under cytokine-poor conditions (B) and differentiation after 8 days of EPO (C) of TF-1 cells expressing a nontargeting shRNA (shControl) or shRNAs targeting TET2 or TET1, as indicated. (D) Proliferation under cytokine-poor conditions of TF-1 cells expressing a TET2 shRNA after pretreatment for 6 days with DMSO,

250 μ M TFMB-(R)-2HG, or TFMB-(S)-2HG. (E) Immunoblot of TF-1 cells stably expressing the indicated IDH1 variants: untreated (-), treated with vehicle (V), or treated with 100 μ M to 1 mM DMOG (as indicated by the thickness of the wedge) for 3 hours before cell lysis. (F and G) Proliferation under cytokine-poor conditions (F) and differentiation after 8 days of EPO (G) of TF-1 cells expressing either IDH1 R132H or an shRNA targeting TET2, and either a nontargeting shRNA (shControl) or shRNAs targeting EglN1. Growth curves show mean values of duplicate experiments $\pm$ SD (error bars). FACS plots show representative results of three independent experiments.

antagonized transformation induced by TET2 knockdown (Fig. 3D). We reasoned that this conundrum might relate to the fact that (*R*)-2HG and (*S*)-2HG have opposing effects on EglN activity, with (*S*)-2HG serving as an inhibitor and (*R*)-2HG, at least in some cellular contexts, serving as an agonist (8). Moreover, we found that TF-1 cells expressing IDH1 R132H were relatively resistant to up-regulation of HIF1 α by the 2OG competitive antagonist dimethylxylglycine (DMOG), suggesting that (*R*)-2HG acts as an EglN agonist in these cells as well (Fig. 3E). We therefore considered the possibility that inhibition of EglN1 by (*S*)-2HG prevents (*S*)-2HG from transforming TF-1 cells. Indeed, we found that knockdown of EglN1 with multiple independent shRNAs abrogated the growth factor independence and restored the differentiation of TF-1 cells transformed by expression of IDH1 R132H or by knockdown of TET2 (Fig. 3, F and G, and figs. S9 and S10).

To determine whether the oncogenic effects of (*R*)-2HG are reversible, we first confirmed that a minimum of four passages in TFMB-(*R*)-2HG were required to render TF-1 cells cytokine-independent, provided that TFMB-(*R*)-2HG exposure was maintained during the cytokine withdrawal period (Fig. 4A). Next, late-passage TF-1 cells treated with TFMB-(*R*)-2HG were passaged out of TFMB-(*R*)-2HG for variable periods before removal of

GM-CSF (Fig. 4B). Interestingly, the amount of time required for reversion of growth factor independence was influenced by the intensity (duration times dose) of TFMB-(*R*)-2HG exposure (Fig. 4, A and B). In contrast, removal of TFMB-(*R*)-2HG rapidly restored the ability of TF-1 cells to differentiate in response to EPO, even after long-term passage in the presence of TFMB-(*R*)-2HG (Fig. 4C). Of note, late-passage TF-1 cells that expressed lower levels of mutant IDH1 (fig. S3B) and produced lower levels of (*R*)-2HG (fig. S7) spontaneously regained the ability to differentiate in response to EPO but retained their ability to proliferate independent of growth factors (fig. S11).

In a complementary experiment, we found that a tool compound (AGI-5198) that specifically blocks 2HG production by IDH1 R132H (fig. S12) (16) restored the ability of IDH1 R132H-expressing TF-1 cells (Fig. 4D) and SCF ER-Hoxb8 cells (Fig. 4F) to differentiate in vitro and abrogated the growth factor independence of IDH1 R132H-expressing TF-1 cells (Fig. 4G and fig. S13). These effects were on-target because they were not observed in cells transformed with TFMB-(*R*)-2HG, rather than IDH1 R132H (Fig. 4, E and H).

Tumor-associated IDH mutations cause many metabolic abnormalities in addition to the accu-

mulation of (*R*)-2HG (9). However, our studies indicate that (*R*)-2HG is sufficient to confer the two hallmarks of leukemia: enhanced proliferation and impaired differentiation. (*R*)-2HG can inhibit many 2OG-dependent enzymes, including various histone demethylases and the 5'-methylcytosine hydroxylase TET2 (6–8). We found that down-regulation of TET2, like high levels of (*R*)-2HG, promotes cytokine independence and blocks differentiation of TF-1 cells. This finding is consistent with the hypothesis that leukemic transformation by mutant IDH is linked to inhibition of TET2 by (*R*)-2HG, thus accounting for the observation that IDH and TET2 mutations both occur in acute leukemia but are mutually exclusive (15).

At intermediate (*R*)-2HG concentrations, TF-1 cells remain cytokine-independent but can once again differentiate. Together with the behavior of TF-1 cells treated with different doses of (*R*)-2HG and their responses to (*R*)-2HG withdrawal, this observation strongly indicates that higher levels of (*R*)-2HG are needed to block differentiation than to confer cytokine independence. The differentiation phenotype may require more profound TET2 inhibition than the growth phenotype. Interestingly, targeted expression of IDH1 R132H in the murine hematopoietic stem cell (HSC) compartment causes gradual

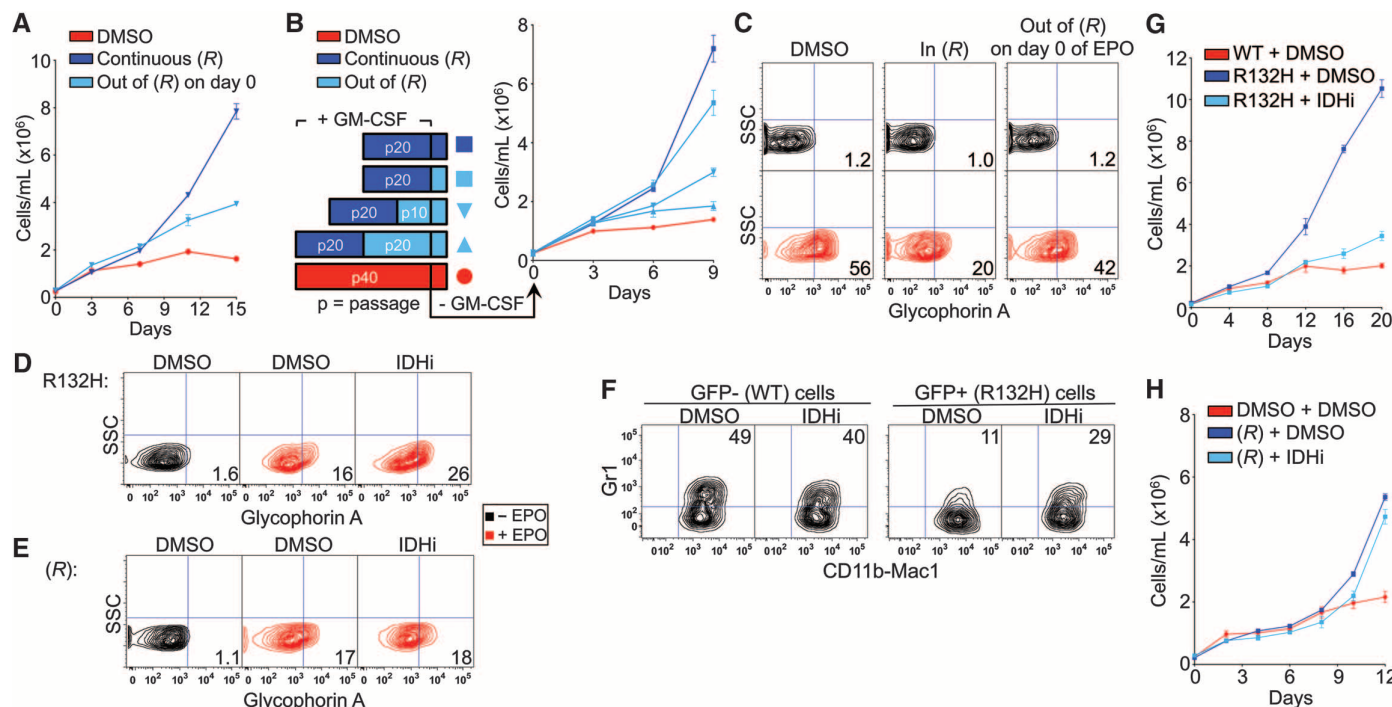


Fig. 4. Transformation by (*R*)-2HG is reversible. (A) Proliferation of TF-1 cells under cytokine-poor conditions after being passaged four times in the presence of DMSO or 250 μ M TFMB-(*R*)-2HG. At the time of GM-CSF withdrawal, TFMB-(*R*)-2HG was either maintained [Continuous (*R*)] or removed [Out of (*R*) on day 0]. (B) Proliferation of TF-1 cells under cytokine-poor conditions after being passaged 20 times in the presence of DMSO or 250 μ M TFMB-(*R*)-2HG and then undergoing wash-out of TFMB-(*R*)-2HG for the periods indicated. (C) Differentiation of TF-1 cells after being passaged 20 times in the presence of 500 μ M TFMB-(*R*)-2HG or DMSO. At the time of EPO administration, TFMB-(*R*)-2HG was either maintained [In (*R*)] or removed [Out

of (*R*) on day 0 of EPO]. (D to F) Differentiation of TF-1 cells (D and E) and SCF ER-Hoxb8 cells (F) transformed with IDH1 R132H (D and F) or TFMB-(*R*)-2HG (E) and then passaged five times in the presence of DMSO or a small-molecule inhibitor of IDH1 R132H (IDHi; 1 μ M). (G and H) Proliferation under cytokine-poor conditions of early passage (p15) TF-1 cells transformed with IDH1 R132H (G) or TFMB-(*R*)-2HG (H) and then passaged five times in the presence of DMSO or a small-molecule inhibitor of IDH1 R132H (IDHi; 1 μ M) before growth-factor withdrawal. Growth curves show mean values of duplicate experiments $\pm$ SD (error bars). FACS plots show representative results of three independent experiments.

expansion of long-term HSCs without any evidence of impaired myeloid differentiation (17). In contrast, mice lacking TET2 exhibit both expansion of their HSC compartment and aberrant myeloid differentiation (18).

Although TET2 appears to be an important target of (R)-2HG, we have not formally proven that TET2 inhibition is necessary, in addition to being sufficient, for transformation by mutant IDH. Inhibition of other 2OG-dependent enzymes by (R)-2HG may enhance or suppress leukemic transformation by mutant IDH. With respect to the latter, the observation that the levels of mutant IDH and (R)-2HG fell over time in TF-1 cells grown under growth factor-rich conditions suggests that high levels of (R)-2HG may be deleterious in certain cellular contexts, perhaps due to inhibition of other 2OG-dependent enzymes that promote cell growth and survival. These considerations might be relevant to the clinical differences between IDH mutant and TET2 mutant myeloid disorders (19).

Leukemic transformation by 2HG is specific to the (R)-enantiomer produced by mutant IDH and not the (S)-enantiomer, even though (S)-2HG is a more potent inhibitor of all of the 2OG-dependent enzymes tested to date, including TET2 (6–8). This conundrum appears to be explained by the differential effects of the two enantiomers on EglN1, with (R)-2HG serving as an agonist and (S)-2HG as an antagonist (8), as well as by our observation that the loss of EglN1 activity blocks transformation by mutant IDH or the loss of TET2. Several studies have indicated that the canonical EglN1 target, HIF, can inhibit HSC and leukemic cell proliferation

(20–26). Interestingly, people living at high altitude—and, hence, presumed to have lower basal EglN1 activity due to chronic hypoxia—appear to have a lower risk of leukemia (27, 28).

The hypothesis that mutant IDH transforms cells by affecting epigenetic marks has raised fears that these changes will not be reversible on a therapeutically relevant time scale. Our studies suggest that the effects of (R)-2HG are reversible, offering hope that compounds that block (R)-2HG production by IDH mutants will benefit patients. Moreover, the relatively rapid reversion of the (R)-2HG transformation phenotypes suggests that the relevant epigenetic marks are more dynamic than previously suspected or that these phenotypes reflect noncanonical functions of enzymes such as TET2. In addition, our findings suggest that pharmacological inhibition of EglN1 might also be useful for the treatment of leukemias that harbor *IDH* or *TET2* mutations.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S13

Table S1

References (29–31)

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ESCRT-III Assembly and Cytokinetic Abscission Are Induced by Tension Release in the Intercellular Bridge

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The last step of cell division, cytokinesis, produces two daughter cells that remain connected by an intercellular bridge. This state often represents the longest stage of the division process. Severing the bridge (abscission) requires a well-described series of molecular events, but the trigger for abscission remains unknown. We found that pulling forces exerted by daughter cells on the intercellular bridge appear to regulate abscission. Counterintuitively, these forces prolonged connection, whereas a release of tension induced abscission. Tension release triggered the assembly of ESCRT-III (endosomal sorting complex required for transport–III), which was followed by membrane fission. This mechanism may allow daughter cells to remain connected until they have settled in their final locations, a process potentially important for tissue organization and morphogenesis.

Long thought to be an unimportant step in cytokinesis (1), abscission is now known to be a complex, tightly regulated process. Recent work has identified several elements required for cutting the cytokinetic bridge (2–6).

These include ESCRT (endosomal sorting complex required for transport), which plays a central role by recruiting the microtubule-severing enzyme spastin (7) and inducing membrane fission (8, 9). Abscission times can vary from 1 hour

after anaphase (10) to 3 hours or more (3), even within similar cell lines. We wanted to identify factors that regulate abscission timing and their associated molecular events.

We first assayed the effect of substrate coating and cell density. Abscission time was similar for cells plated on glass and at high density on fibronectin, but it increased with decreasing cell density (Fig. 1, A and C, and movie S1). These results exclude a direct effect of substrate coating but suggest that abscission time is shorter when cells are closely packed.

To discriminate between effects caused by cell spreading or movement and those caused by cell-cell contact, we plated single cells on disk-shaped fibronectin micropatterns. Abscission time increased with increasing disk size (Fig. 1, B and D, and movie S2). We obtained similar results with thin bar-shaped micropatterns that imposed a given maximal separation on the daughter cells but restrained their spreading area

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Fig. 1. Low spatial confinement of daughter cells delays abscission. **(A and B)** Representative images of daughter cell morphology (visualized by α -tubulin–EGFP signal). Insets show 4 $\times$ zoom on the intercellular bridge. Scale bar, 10 μ m. **(C and D)** Quantification of abscission time. Each dot represents one daughter cell doublet; means are shown as horizontal bars. Fn., fibronectin. *** $P < 0.0001$ (Kruskal-Wallis test); n.s., not significant.

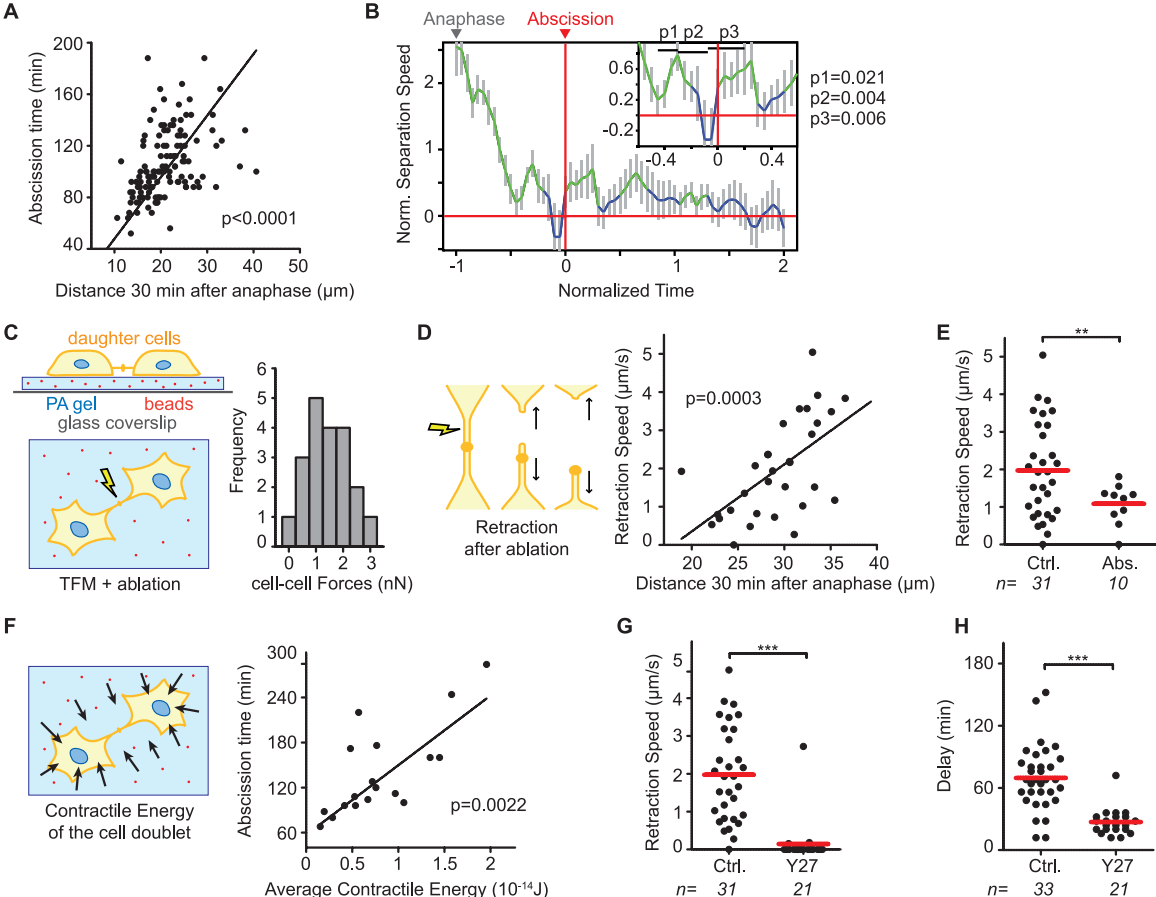
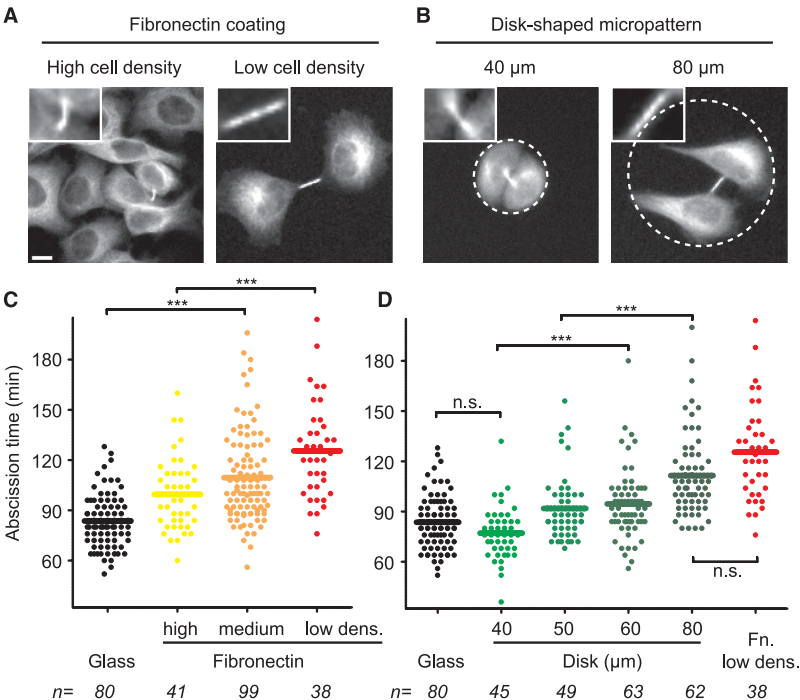


Fig. 2. Tension in the intercellular bridge regulates abscission time. **(A)** Correlation between initial cell separation and abscission time; $n = 126$. **(B)** Separation speed as a function of time (means $\pm$ SEM). Green line segments indicate separation speed significantly above 0 (t test); $n = 35$. **(C)** Distribution of forces between daughter cells (TFM and laser ablation of the bridge); $n = 20$. **(D)** Correlation between bridge tension (retraction speed) and initial separation

of daughter cells; $n = 31$. **(E)** Bridge tension just prior to abscission (Abs.) compared to randomly chosen bridge (Ctrl.). **(F)** Abscission time as a function of the contractility of the daughter cell doublet; $n = 18$. **(G)** Bridge tension for cells treated by Y27632. **(H)** Delay between drug treatment and abscission. Significance: [(A), (D), and (F)] P value of Spearman test (linear fit); [(E), (G), and (H)] *** $P < 0.005$, *** $P < 0.0001$ (t test with Welch correction).

(fig. S1). Thus, abscission time was not modulated by cell-cell contact or cell spreading, but rather by the capacity of daughter cells to move apart from each other after division. Counterintuitively, the more cells could move apart, the longer abscission was delayed. To confirm this, we measured cell separation speed during postmitotic respreading and found that the faster the cells separated, the longer the abscission time (Fig. 2A).

Faster-separating cells displayed delayed or impaired microtubule severing, a phenotype similar to small interfering RNA (siRNA) knock-down phenotype of spastin (11) (figs. S2 and S3 and movies S3 and S4). Thus, it seems that when daughter cells respread after anaphase, they pulled on the cytokinetic bridge as they separated, delaying the severing of microtubules by spastin. This model predicts that cells that either do not pull on the bridge or stop pulling undergo ab-

scission rapidly. Careful examination of bridge morphology and use of specific micropatterns (11) suggested that tensed (straight) bridges were indeed correlated with longer abscission time. Often the two daughter cells transiently moved toward one another, bending the bridge, with abscission following shortly thereafter (fig. S4, C and D). When we quantified this behavior, we found that cells stopped moving apart about 10 min before abscission (Fig. 2B).

To directly measure forces exerted on the connecting bridge, we combined traction force microscopy (TFM) and laser ablation (11–13) (fig. S5A). This showed that the bridge was bearing forces in the nanonewton range (1.4 ± 0.2 nN; Fig. 2C) (14). Retraction speed of the bridge after ablation (11, 15) (Fig. 2D, fig. S6, and movie S5) revealed similar forces. The daughter cells' separation speed and bridge tension were strongly correlated, showing that faster-separating daugh-

ter cells were pulling more strongly on their bridges (Fig. 2D). We identified bridges on the brink of abscission by imaging microtubules (fig. S7A). Such bridges were more relaxed than average bridges (Fig. 2E), confirming that bridge tension decreased before abscission. Thus, counterintuitively, tension in the cytokinetic bridge delays abscission.

This contradicts an existing model of abscission, which is based on the measurement of traction forces exerted by cells on their substrate after division (1). We thus performed similar experiments but using TFM, and found that cells displayed a high contractility after division and pulled strongly on the substrate (fig. S5B and movies S6 and S7). Nonetheless, the higher these forces were, the longer the abscission time (Fig. 2F).

Cell contractility might thus be responsible for the high force exerted by daughter cells on their connecting bridge. We decreased the cells' contractility by treating them with the Rho-associated protein kinase (ROCK) inhibitor Y27632. This strongly reduced both bridge tension (Fig. 2G and fig. S8A) and abscission delay (Fig. 2H). Thus, tension in the cytokinetic bridge could be caused by cell contractility, and reducing bridge tension induced abscission.

One potential source of bridge tension is membrane tension, which can be estimated by pulling a membrane tether with a laser trap (16) (fig. S9, A and B, and movie S8). The tether force was on the order of tens of piconewtons, which corresponds to a contribution of membrane tension to the force applied on the bridge on the order of 0.4 to 0.8 nN (11). Thus, membrane tension could make an important contribution to bridge tension. Membrane tension was low in cases where cells had short abscission times (on glass or treated with Y27632; fig. S9, C and D). It was high when cells had long abscission times (on fibronectin; fig. S9C).

Together, these results suggest the following model: When cells are free to move apart after division, high contractility and motility correlate with a high membrane tension, generating a nanonewton pulling force on the cytokinetic bridge, which delays abscission. When cells stop moving apart, bridge tension drops, triggering abscission. To test our model, we reduced bridge tension by artificially cutting the bridge on one side. We followed microtubules in the remaining half-bridge after laser ablation; the central part was stable, and a cut on the other side was observed shortly thereafter (Fig. 3, B to D, and movie S9), as happens during normal abscission (Fig. 3A).

To rule out a nonspecific effect of ablation on microtubule disassembly, we depleted key abscission proteins by siRNA: spastin, essential for microtubule severing, and CHMP2A (charged multivesicular body protein 2A), an essential component for ESCRT-III complex assembly (fig. S10A and movie S10). Ablation in spastin-depleted cells led to the characteristic ESCRT-III-dependent pinching of the bridge on the other side, but this

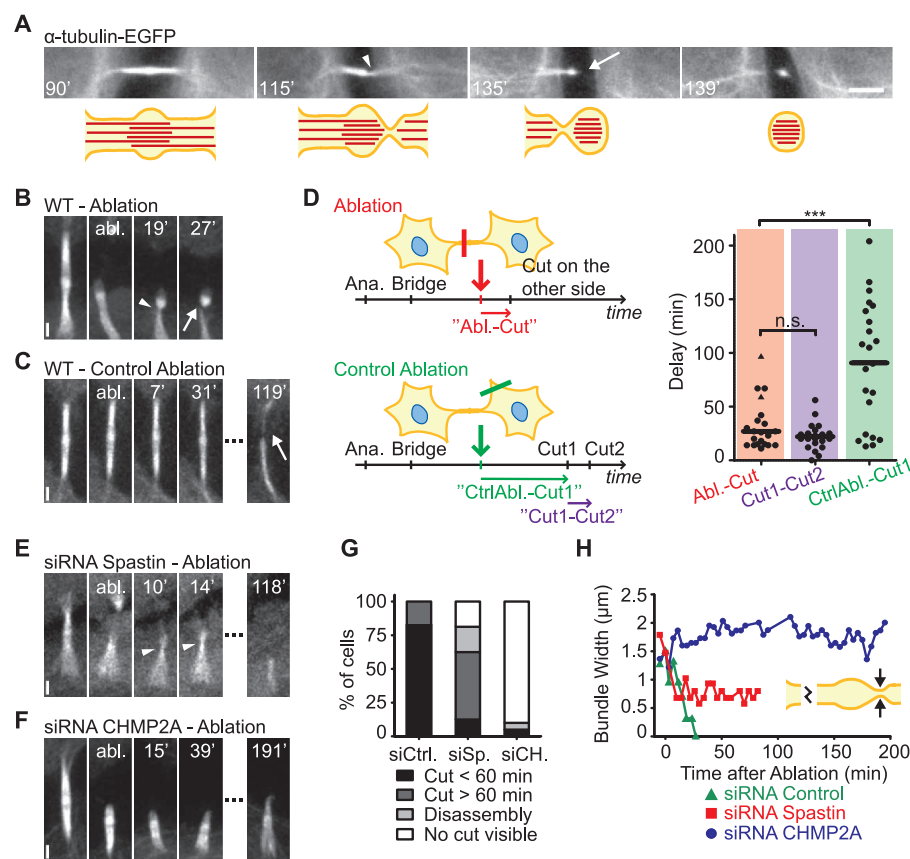


Fig. 3. Artificial release of bridge tension triggers abscission. (A) Time-lapse sequence of the abscission process. (B and C) Ablation on one side of the bridge (B) and control ablation (C); the leftmost image is before ablation (abl.). (D) Quantification of delays between ablation and the cut on the other side of the bridge (red, $n = 18$), between the first cut and the second cut in control cells (purple, $n = 23$), and between the control ablation and the abscission (green, $n = 21$). $***P < 0.0001$ (Kruskal-Wallis test). (E and F) Ablation performed respectively on siRNA spastin-treated cells and siRNA CHMP2A-treated cells. (G) Percentage of cells showing abscission after the bridge ablation with a delay shorter than 60 min (black) or longer than 60 min (dark gray), a slow disassembly of the microtubule bundle (light gray), or no abscission for more than 180 min (white) for siRNA control-treated ($n = 17$), siRNA spastin-treated ($n = 16$), and siRNA CHMP2A-treated ($n = 20$) cells. (H) Evolution of the bundle width after ablation. Scale bars, 10 μ m (A), 2 μ m [(B), (C), (E), and (F)]; time is minutes after anaphase or after ablation; arrowhead shows microtubule bundle pinching; arrow shows abscission.

was not followed by microtubule severing (Fig. 3, E, G, and H, and fig. S7B). Thus, the induced microtubule severing was spastin-dependent and not nonspecific. Ablation in CHMP2A-depleted cells did not lead to any pinching and was not followed by any microtubule severing (Fig. 3, F to H). This finding was expected, because spastin is targeted to the bridge by ESCRT components (7). Thus, ablation of one side of the bridge induces the regular ESCRT-III-spastin abscission pathway on the other side.

To investigate this point, we tagged CHMP4B, one of the ESCRT-III components that forms the late cone preceding abscission, with green fluorescent protein (GFP). As previously de-

scribed (8, 9), CHMP4B-GFP was recruited to both sides of the midbody, forming two narrow bands. One of the bands then extended on one side, concomitant with a narrowing of the bridge on that side and with a severing of microtubules (Fig. 4A); a similar process then took place on the other side (movie S11).

We also performed siRNA knockdowns of spastin and CHMP2A in these cells and observed the expected phenotypes (fig. S10B) (11). To identify the stage of bridge cleavage triggered by the release of tension, we performed laser ablation experiments at different stages, determined by CHMP4B-GFP localization at the bridge. Ablations performed after CHMP4B-GFP recruit-

ment to the midbody resulted in assembly of the conical structure on the other side 10 min later, concomitant with pinching and microtubule severing (Fig. 4, B and C, and movie S12). Ablations performed on bridges before any CHMP4B-GFP was visible led to transient recruitment of CHMP4B-GFP but no subsequent cone formation or abscission (Fig. 4D).

Ablations in spastin-depleted cells led to the formation of CHMP4B-GFP conical structures and pinching with normal timing, but this was not followed by microtubule severing, whereas ablation in CHMP2A-depleted cells did not trigger any CHMP4B-GFP conical structure assembly nor microtubule severing (Fig. 4E). Thus,

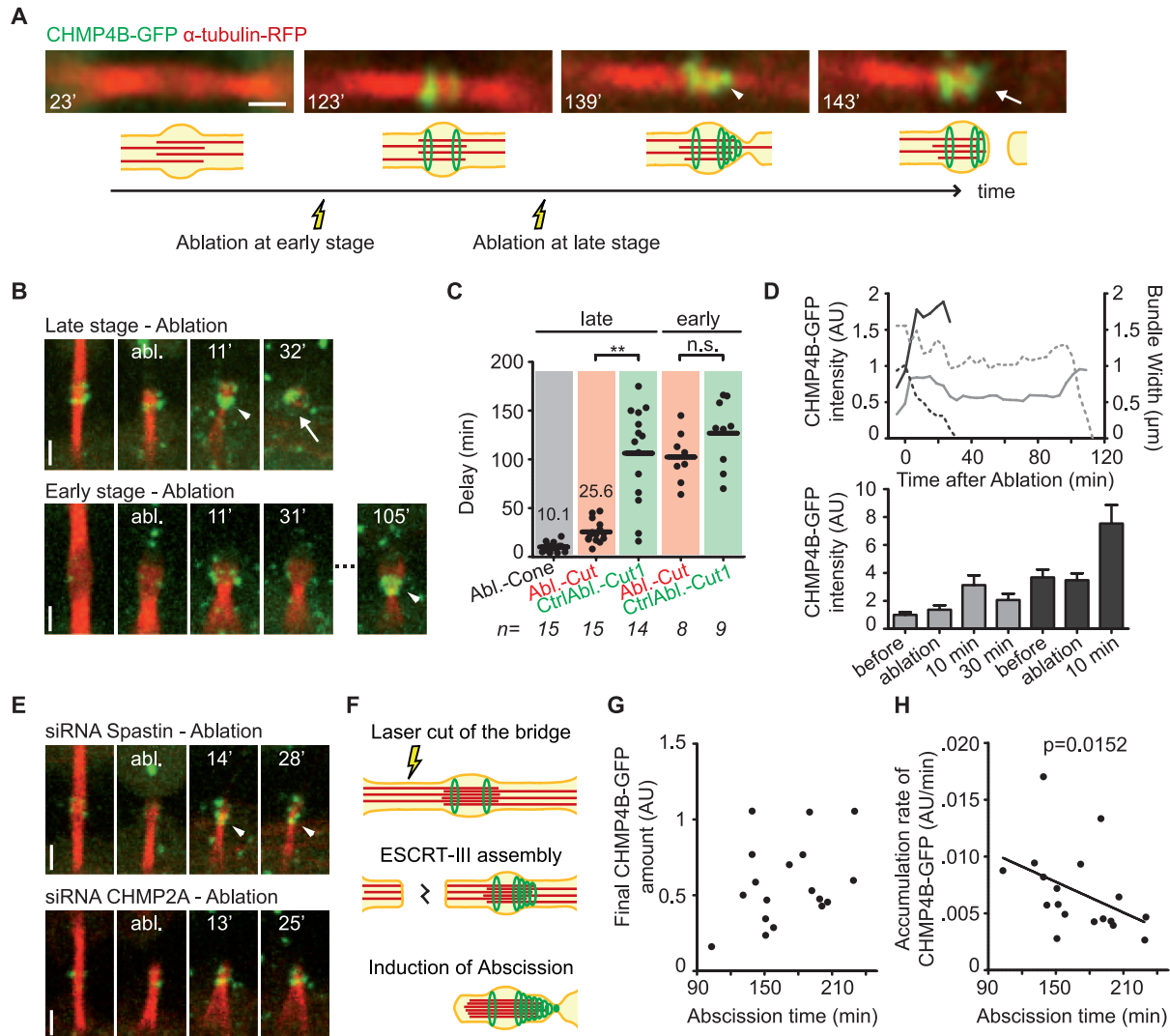


Fig. 4. ESCRT-III assembly is regulated in time by forces exerted on the bridge. **(A and B)** Time-lapse sequence of control bridge cleavage **(A)** and ablated bridge **(B)**. Leftmost image is before ablation (abl.). **(C)** Quantification of delays between ablation and formation of the ESCRT-III cone (gray), between ablation and abscission (red), and between control ablation and abscission (green). Late (left) and early (right) stages were quantified. $**P < 0.05$ (Kruskal-Wallis test). **(D)** Top: Representative curves of CHMP4B accumulation at the midbody (plain curves, left axis) and microtubule bundle width (dashed curves, right axis) after ablation at early stage (gray) and late stage (black). Bottom: CHMP4B accumulation at the midbody (means $\pm$ SEM)

before ablation (before), just after ablation (ablation), and 10 or 30 min after ablation (early $n = 6$, late $n = 12$). AU, arbitrary units. **(E)** Time lapse of bridge cleavage in ablated siRNA-treated cells. **(F)** The proposed model. Cell membrane (yellow), microtubules (red), and ESCRT-III (green) are shown. **(G)** Final amount of CHMP4B accumulated at the midbody before abscission. **(H)** Accumulation rate of CHMP4B at the midbody during cytokinesis as a function of abscission time; $n = 18$. P value of Spearman test (linear fit) is shown. Scale bars, 2 μ m [(A), (B), and (E)]. Time is minutes from anaphase or ablation; arrowhead shows microtubule bundle pinching and formation of the ESCRT-III conical structure; arrow shows abscission.

tension release after ablation specifically induces the very last step of abscission, the assembly of the ESCRT-III conical structure (Fig. 4F).

Our results have allowed us to identify a major source of variability in abscission time: the forces exerted by daughter cells on the cytokinetic bridge. To respread after mitosis, daughter cells pull on their substrate. Depending on their environment, part of this force is transmitted to the cytokinetic bridge, resulting in delayed ESCRT-III assembly and thus in delayed abscission (fig. S11). We measured separation speed in another cell line showing longer abscission time and found a similar relationship between these two parameters (fig. S12) (11). This suggests that the variability in abscission time reported in different cell lines can be explained simply by differences in postmitotic separation speed.

How do forces acting on the bridge delay ESCRT-III assembly? Membrane tension (17) or local curvature (18) could directly affect ESCRT assembly. Indeed, a recent physical model of ESCRT-III assembly implies that membrane tension could interfere with the process (19). Alternatively, mechanically induced signaling processes might be involved; for example, RhoA and Rac1 regulation are involved both in mechanotransduction (20, 21) and abscission (22).

Other mechanisms involved in the regulation of abscission were reported to rely on the regulation of ESCRT complexes at the midbody (23, 24). Here, we also found that bridge tension delays ESCRT-III assembly. Quantifying CHMP4B-GFP recruitment at the midbody showed that

although the final level of accumulation at abscission was not correlated with abscission time, the rate of accumulation was lower in cells in which abscission was delayed (Fig. 4, G and H, and fig. S10C).

We propose that cells possess a sensory mechanism, potentially involving membrane tension, that delays abscission by preventing ESCRT-III accumulation and assembly as long as forces are exerted on the cytokinetic bridge. This mechanism would allow daughter cells to reestablish connections after mitosis—thus preventing tissue rupture or cell loss—and achieve correct relative positioning.

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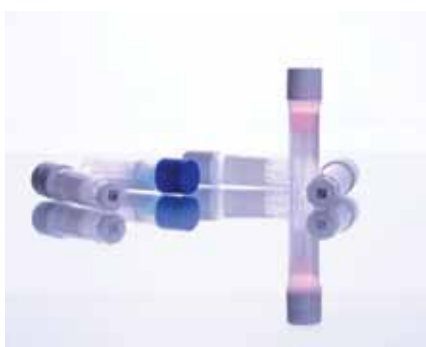
Acknowledgments: We thank D. Gerlich for the Hela Kyoto α -tubulin-EGFP histone2B-mCherry cell line and the α -tubulin plasmids; I. Poser and A. Hyman for the CHMP4B-GFP cell line; A. Roux, A. Echard, N. Minc, C. Janke, and members of the Piel lab for critical reading of the manuscript and C. Ainsworth for editing it; L. Sengmanivong, V. Fraissier, the Nikon Imaging Center, and the PICT-IBiSA of the Institut Curie for technical support in microscopy; C. Sykes; and E. Terriac and A. Jimenez for helpful discussions. Supported by a French ministerial fellowship (J.L.-J.), a Fondation pour la Recherche Medicale fellowship (J.L.-J. and P.M.), a Ligue Contre le Cancer grant (M.P.), ANR-11-JSV5-0002 (T.B.), and Fondation Nanosciences RTRA (M.B.). The data are contained in the manuscript and the supplementary materials.

Supplementary Materials

www.sciencemag.org/cgi/content/full/339/6127/1625/DC1

Materials and Methods
Supplementary Text
Figs. S1 to S14
Movies S1 to S12
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Takao Shimizu

Novel Approaches to Translational Medical Research

Translational research has recently become a stronger focus for the University, with the goal of solving real-life problems that positively impact society as a whole. “To complement our successes in interdisciplinary research we are initiating major programs to transfer the seeds of our basic research into devices, protocols, and products for the benefit of society,” explains **Takao Shimizu**, a biochemistry professor at Todai's Medical School and executive vice president of the University. Examples of internationally acclaimed and innovative programs and infrastructure for translational research include the Translational Research Initiative (TRI) and Open Innovation Center for Drug Discovery (OCDD).

The Translational Research Initiative

The TRI aims to bring about a paradigm shift in health care technologies. “Our mission is to translate basic research at Todai into practical healthcare applications,” says **Takashi Kadowaki**, the director of the University of Tokyo Hospital and head of TRI. “We have experts from the arts, sciences, engineering, and medicine, all collaborating at the 22nd Century Medical and Research Center [CMRC].”

Twenty departments make up the CMRC, each with an endowment of US\$500,000 per year for 10 years. “This is the largest concentration of endowed departments in Japan,” says Kadowaki.

From Diabetes to Biomedical Engineering

Kadowaki, who manages the TRI and treats hospital patients, also conducts research on diabetes and metabolic diseases. “Changes in dietary habits since the mid-1950s have led to a 35-fold increase in diabetic patients in Japan,” he says. “In 2007, Japan had an astonishing 8.9 million confirmed diabetes cases.” In 2003, Kadowaki's group discovered that downregulation of the so-called adiponectin receptor (Adipor) was a major cause of obesity-related insulin resistance in type 2 diabetics. “We are now in the preclinical stage of translational medical research on ‘adiponectin receptor activators’ (ARAs) to counteract insulin resistance. Treating diabetes will also reduce the impact of associated ailments such as kidney disease, stroke, and ischemic heart disease.”

Engineers also play an important role in research at TRI. “Our teams of leading biomedical engineers interact frequently with their counterparts in translational medicine research,” says **Ichiro Sakuma**, a leading researcher at the Center for Medical System Innovation (CMSI) at TRI.

Other research at CMSI includes the fabrication of supramolecular nanocarriers for drug delivery (Kazunori Kataoka), the synthesis of artificial polymer-based cell membranes (Kazuhiko Ishihara), the regeneration of bone and cartilage for oral and maxillofacial surgery (Tsuyoshi Takato), the synthesis and medical applications of photo-responsive biomolecules (Teruyuki Nagamune), and the development of lab-on-a-chip and other microfluidic devices (Takehiko Kitamori).

New Research Facilities

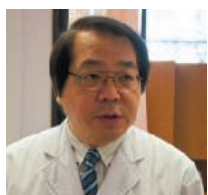
Research infrastructure is critical for effective translational medical research. The ‘Phase One Unit,’ a new treatment and research unit, has recently been added to the University of Tokyo Hospital Clinical Research Support Center (UT-CresCent). The unit has 12 beds, magnetic resonance imaging and positron emission tomography-computed tomography scanners, and state of the art medical diagnostic equipment. “These facilities will enable us to reduce the time taken to test potential drugs for the treatment of ailments including Alzheimer's disease,” explains **Takashi Moritoyo**, head of the new unit.



Takashi Kadowaki



Ichiro Sakuma



Takashi Moritoyo



GREEN: Asano Campus; GOLD: University Hospital
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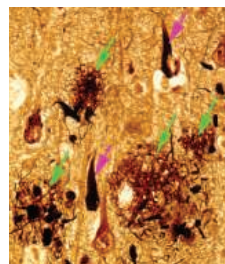
Takeshi Iwatsubo

Preventative Medicine: Predicting Alzheimer's Disease

Japan—like many other industrial nations—is dealing with a rapidly aging population. In particular, the limitations of present-day medical care and social support infrastructure make coping with increases in Alzheimer's disease (AD) a challenge. **Takeshi Iwatsubo** in the Department of Neuropathology is the principle investigator of the Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI). “J-ADNI was launched in 2007 to establish a complete set of biomarkers to predict the onset of AD, in particular the progression from mild cognitive impairment to AD,” explains Iwatsubo. “The goal is to develop disease-modifying drug treatments.”

As of 2012, J-ADNI had tested approximately 600 subjects at 38 clinical sites throughout Japan and has collected a plethora of MRI, brain activity, pathology, and biomarker data. These results will be analyzed for patterns indicative of disease progress and also used to track patient response to treatment.

Researchers at J-ADNI are collaborating with 11 major drug manufacturers to identify and test promising therapies, including Japan's Takeda Pharmaceutical Company Limited, from which Todai obtained the license for TAK-070—a β -secretase inhibitor—for phase 1 clinical trials scheduled to start in March 2013. “There are at least two million AD patients in Japan alone,” says Iwatsubo. “We expect preemptive medical diagnosis in preclinical AD to improve the quality of life for millions of people worldwide.”



Takeshi Iwatsubo

Researchers at Todai have contributed to understanding the causes of Alzheimer's disease through the discovery of amyloid β 42 in senile plaques (green arrows) and tau protein in neurofibrillary tangles (pink arrows).

Basic Research Seeds for Translational Medical Research



Kohei Miyazono

Department of Molecular Pathology

Research into the autocrine signaling of the cytokine transforming growth factor beta ($\text{TGF-}\beta$) has led to the discovery that it can regulate the ability of so called glioma-initiating cells to produce tumors in the brain. Importantly, Miyazono and colleagues found that $\text{TGF-}\beta$ receptor inhibitors could act as effective antitumor agents.



Yasuteru Urano

Department of Chemical Biology and Molecular Imaging

Urano has

developed a method for rapid in vivo cancer detection using a novel γ -glutamyltranspeptidase-activated fluorescent probe. The probe ‘switches on’ when taken up by tumor cells. His research was featured on the 23 November 2011 cover of *Science Translational Medicine*.



Hiroshi Takayanagi

Department of Immunology

Takayanagi and colleagues initiated osteoimmunological studies into the molecular mechanisms of bone destruction in arthritis. They found that an antibody against the proangiogenic semaphorin 4-D molecule promoted bone formation, but did not impact resorption. Related patents have been licensed to a U.S. company that plans to perform clinical trials on its use for the treatment of arthritis, osteoporosis, and cancer.



Clockwise from left: Hidenori Ichijo, Takayoshi Okabe, Hirotatsu Kojima

The Open Innovation Center for Drug Discovery

Hidenori Ichijo is director of the Open Innovation Center for Drug Discovery (OCDD) and with his colleagues, Takayoshi Okabe and Hirotatsu Kojima, manages the OCDD, a central facility for drug screening in Japan. In contrast to other institutions providing complete screening services, the OCDD offers facilities and training but requires researchers to conduct screening themselves, giving them more control over their own research.

“We store a huge range of chemical compounds—approximately 210,750 as of 2012—that are available for screening to identify new drugs, including so-called orphan

drugs for treating rare diseases,” explains Ichijo. In addition to shipping samples to researchers around Japan, the OCDD also provides advice and the use of its facilities. “Through the end of 2012, we have accepted 646 applications for use of the OCDD library, provided 3.6 million samples, and held 629 advisory meetings,” says Okabe.

A recent research highlight is an important finding by Ichijo concerning amyotrophic lateral sclerosis (ALS)—a rare motor neuron disease. Ichijo and colleagues used the OCDD library to tease out some of the biochemical pathways in ALS. “This was the first study focusing on the general relationship between superoxide dismutase-1 [SOD1] mutants and the onset of ALS,” says Ichijo.

Looking Ahead and Abroad

“Education and research are evolving at Todai,” states executive vice president Shimizu. “President Junichi Hamada has stated the need to ‘change the whole education system,’” he continues. “So we will start our academic year in September to synchronize with overseas universities.” This shift symbolizes the emphasis that Todai places on internationalization, taking an outward-looking stance as the University meets, in Shimizu's words, “the daunting challenges facing the next generation.” Shimizu also strongly believes “that student and faculty diversity and mobility are vital for the university to nurture students able to take on those challenges.”

ADDITIONAL RESOURCES

22nd Century Medical and Research Center
www.h.u-tokyo.ac.jp/english/center22/index.html

Center for Disease Biology and Integrative Medicine (CDBIM)
www.cdbim.m.u-tokyo.ac.jp/english/center.php

Cooperative Unit for Medicine and Engineering Research
www.ikourenk.umin.jp

Graduate Program for Leaders in Life Innovation (GPLLI)
square.umin.ac.jp/gplli/

Japanese Alzheimer's Disease Neuroimaging Initiative
www.j-adni.org/etop.html

Open Innovation Center for Drug Discovery
www.ocdd.u-tokyo.ac.jp/index_e.html

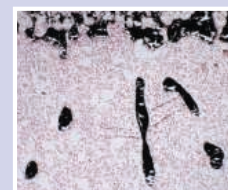
Translational Research Initiative
tri.u-tokyo.ac.jp/en/

University of Tokyo Hospital
www.h.u-tokyo.ac.jp/english/

University of Tokyo Hospital Clinical Research Support Center (UT-CresCent)
www.cresc.h.u-tokyo.ac.jp/en/index.html

Todai Research
www.u-tokyo.ac.jp/en/todai-research/

University of Tokyo
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Hiroshi Takayanagi



Augmented bone formation (black) in a *Sema4d*^{-/-} mutant (below) in contrast to a wild type mouse (above).

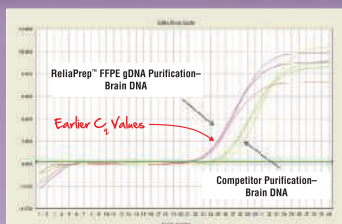
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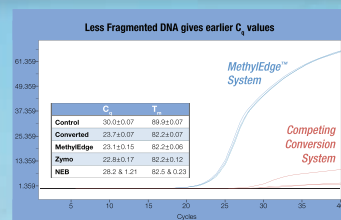
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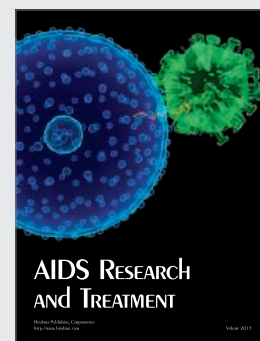
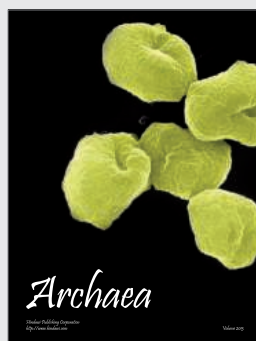
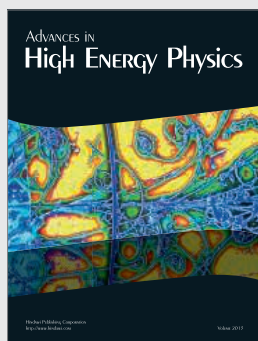
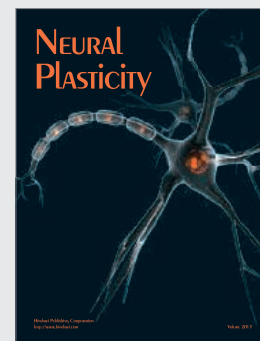
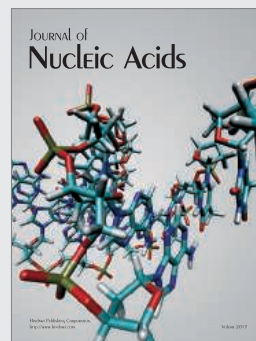
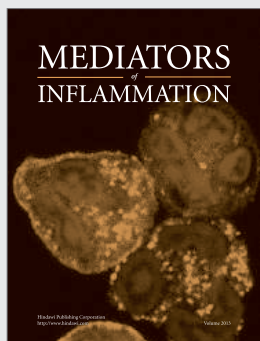
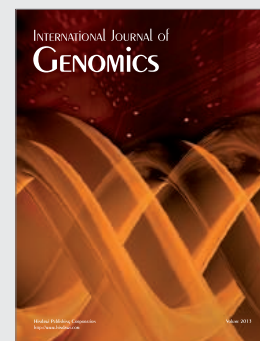
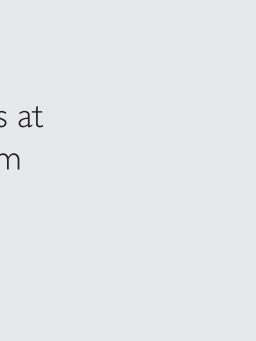
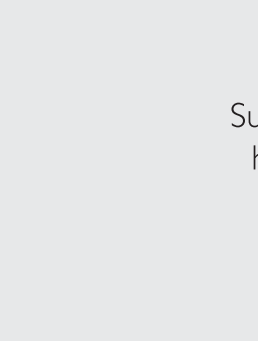
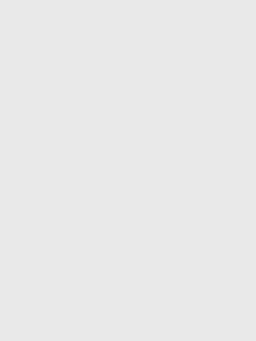
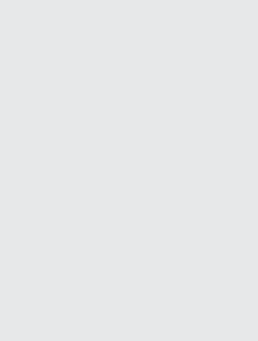
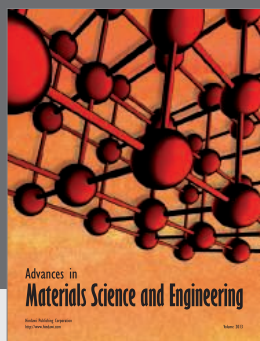


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2012 Cozzarelli Prize Recipients

CLASS I: PHYSICAL AND MATHEMATICAL SCIENCES

Water, plants, and early human habitats in eastern Africa

Clayton R. Magill, Gail M. Ashley, and Katherine H. Freeman

(2013) PNAS 110:1175–1180

CLASS II: BIOLOGICAL SCIENCES

Eight pairs of descending visual neurons in the dragonfly give wing motor centers accurate population vector of prey direction

Paloma T. Gonzalez-Bellido, Hanchuan Peng, Jinzhu Yang, Apostolos P. Georgopoulos, and Robert M. Olberg

(2013) PNAS 110:696–701

CLASS III: ENGINEERING AND APPLIED SCIENCES

Point process modelling of the Afghan War Diary

Andrew Zammit-Mangion, Michael Dewar, Visakan Kadirkamanathan, and Guido Sanguinetti

(2012) PNAS 109:12414–12419

CLASS IV: BIOMEDICAL SCIENCES

Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling

Xiaojun Lian, Cheston Hsiao, Gisela Wilson, Kexian Zhu, Laurie B. Hazeltine, Samira M. Azarin, Kunil K. Raval, Jianhua Zhang, Timothy J. Kamp, and Sean P. Palecek

(2012) PNAS 109:E1848–E1857

CLASS V: BEHAVIORAL AND SOCIAL SCIENCES

Evolution of music by public choice

Robert M. MacCallum, Matthias Mauch, Austin Burt, and Armand M. Leroi

(2012) PNAS 109:12081–12086

CLASS VI: APPLIED BIOLOGICAL, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES

***Arabidopsis* synchronizes jasmonate-mediated defense with insect circadian behavior**

Danielle Goodspeed, E. Wassim Chehab, Amelia Min-Venditti, Janet Braam, and Michael F. Covington

(2012) PNAS 109:4674–4677

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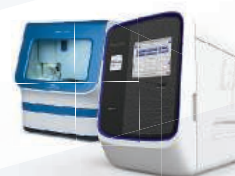
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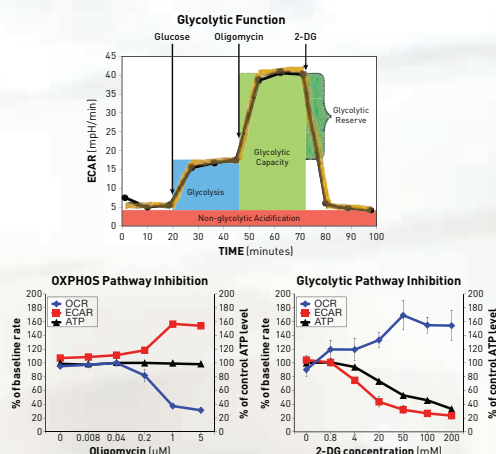


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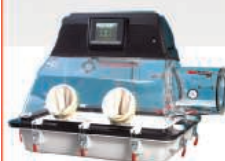
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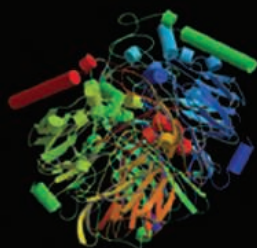
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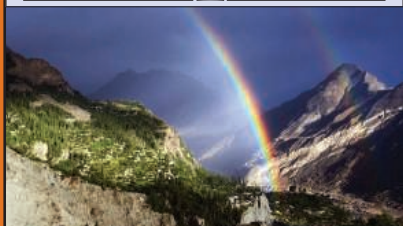


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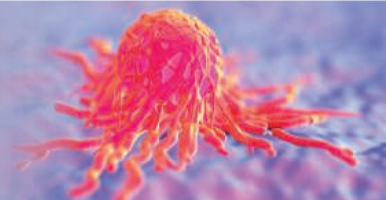
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In This Issue

Once upon a time, there were only two career options for life scientists interested in cancer research: academia or industry. Each had its own culture defined by unique core objectives, and each had distinctive ways of managing systems of research, reputations, and rewards. Freshly minted postdocs may think they can still only do one or the other, but a new paradigm has emerged. Multidisciplinary collaborations across sector and scholarship lines are creating opportunities for ambitious cancer-fighting careers at the junction of the educational and business sectors.

See the full story on page 1633.

Upcoming Features

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Once upon a time, there were only two career options for life scientists interested in cancer research: academia or industry. Each had its own culture defined by unique core objectives, and each had distinctive ways of managing systems of research, reputations, and rewards. Freshly minted postdocs may think they can still only do one or the other, but a new paradigm has emerged. Multidisciplinary collaborations across sector and scholarship lines are creating opportunities for ambitious cancer-fighting careers at the junction of the educational and business sectors. **By Alaina G. Levine**

Cancer research is broadly guided by the mission to find treatments and cures, but the way academic and industrial scientists conduct their investigations often diverge. One of the most notable differences is the driving force of the research itself. Academic research seeks to unveil a basic understanding of science, and is “deductive, granular, and mechanistic,” describes **David Sidransky**, director of the Head and Neck Cancer Research Division at Johns Hopkins University (JHU) School of Medicine in Baltimore, Maryland. There is freedom to explore theoretical ideas often with little concern about timelines.

Industry, on the other hand, is very goal-oriented, and even basic research is geared towards being translational, says Sidransky. Priority is placed on developing commercializable cancer therapies. Whereas in academia a typical investigation might entail determining “the affinity of an antibody to its receptor and the effects on signaling,” the commercial question, he clarifies, would focus more on “how antibody binding affects other targets for toxicity and on the overall cellular clinical response to the antibody, rather than on the details of the signaling pathways.”

Scaling up the technology and manufacturing multiple versions of a product are discussed early on in industrial projects, says **Scott Eliasof**, vice president of research at Cerulean Pharma, a Cambridge, Massachusetts-based company with a focus on designing tumor-targeted nanopharmaceutical therapies. “Industry scientists focus more on the nuts-and-bolts of moving a project into the clinic, a skill set not often found in academia.”

“Industry and academia have two different motives for research,” says **Benjamin Y. Clark**, an assistant professor in the Maxine Goodman Levin College of Urban Affairs at Cleveland State University, in Ohio. The private sector is motivated by product development and profit, whereas academia is motivated by intellectual curiosity, he notes.

“They each have a different bottom line and financial support comes from different places.”

It is no surprise that Big Pharma won’t fund a decade’s worth of research on a molecule that can’t be molded into a cancer therapy. “In the private sector you are time-constrained and not resource constrained, while it is the opposite in academia,” says **Jennifer Malin**, an associate clinical professor at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA), and the managing medical director of oncology for WellPoint, an Indianapolis, Indiana-based parent company of 14 health insurance plans.

Indeed, resource availability and diversity are cited differences between the two realms. In industry, especially in large, older corporations, resources are often plentiful. “Big Pharma has an almost infinite amount of resources and lots of expertise,” says Eliasof. However, while a smaller company like his, which only has 33 employees, may not have a wealth of financial resources from which to partake, there is more opportunity to affect agendas and decide company priorities, he notes. This element in particular can be very appealing to academic scientists pondering a move to industry.

“Depending on the type of cancer research, [scientists] can become quite siloed in academia,” says Malin. “But it’s impossible to work in silos in the private sector.” Companies rely on collaboration across disciplines such as cancer biology, oncology, cellular biology, chemistry, and biotechnology. “Industry scientists often work closely with **continued**”

“Work environments are becoming more diverse, especially in academia. If [researchers] want to get anything done, they have to work across disciplines.”

—Benjamin Y. Clark

Jay Strum



Benjamin Y. Clark



Upcoming Features

Biotech and Pharma:
Forging Academic Collaborations—April 12
Regional Focus: Wales—April 26
Diversity (online only)—June 7



Linda H. Malkas

“Young people today...need to learn that the old cowboy way of doing science where you work by yourself is gone.”
—Linda H. Malkas

oncologists who treat patients, and can therefore have a better understanding of what is needed to make a difference in cancer patients’ lives,” says Eliasof. “To me, this is one of the most important differences between industry and academia and, in fact, is why I moved to industry.”

The interdisciplinary nature of the private sector extends beyond research. Scientists often end up working with professionals in regulatory affairs, quality control, and even marketing. “It spans

the whole continuum of disciplines, whereas in academia ‘multidisciplinary’ means it spans the sciences only,” says Malin, although, more and more, this is changing.

Team-based projects are a mark of industrial cancer research. “The notion that you can work in a larger team and provide improved value for the patient is a central goal for our company,” says **Samuel Levy**, chief scientific officer of Genomic Health, a molecular diagnostics oncology company based in Redwood City, California. Teamwork is essential because in industry “people are evaluated by how well your team does,” says Malin. In fact, training to be a good manager is often a fundamental part of serving as a researcher in industry, she continues.

And then there’s the issue of recognition. Eliasof sums up the spirit of each arena: “Academic scientists are rewarded for publishing novel, cutting-edge research,” he explains, “but the novelty may or may not be practical at the end of the day. In contrast, industry scientists are rewarded for identifying scientifically and financially pragmatic projects and bringing them to the clinic, where they can help cancer patients.”

Mastering Coveted Skills

In most cases, many of the skill sets are the same for cancer researchers in academia and industry, says **Linda H. Malkas**, who serves as the deputy director of basic research, co-leader of the molecular oncology program, and a professor of molecular and cellular biology at City of Hope (COH) in Duarte, California. Leadership potential, creativity, and oral and written communication skills are qualities that both academic and industrial scientists can master in their own environments.

“If you look at the best academic scientists, they are great storytell-

ers,” says Eliasof. Researchers in industry also have to be able to effectively articulate missions and complex scientific problems, although their stakeholders and audiences tend to be more diverse.

Eliasof expresses concern that team building, a universally coveted skill, sometimes doesn’t manifest itself well in universities. “When I bring people in right from academia sometimes they haven’t learned how to function efficiently on teams,” he explains.

And there are other skills that might not be as easy to learn while in an academic setting. Malkas attests that scientists can hone special skills by working alongside clinicians and patients, which is an opportunity that university and business enterprises don’t always provide. At City of Hope, early career scientists learn to think translationally and to always consider how they can apply their knowledge to affect cancer treatment, she says.

Trends and Shifts in Cancer Research

But overall, the culture within both academia and industry is starting to change. “Work environments are becoming more diverse, especially in academia,” says Clark. “If [researchers] want to get anything done, they have to work across disciplines.” **Jay Strum**, president of G1 Therapeutics, a Chapel Hill, North Carolina-based enterprise that focuses on small molecule therapies, regularly collaborates with university researchers and has noticed, for example, that statisticians are playing larger roles in academic cancer research teams. “The trend is moving towards better designed experiments [that incorporate] statistics earlier on,” he says.

Moreover, initiatives like National Science Foundation- and National Cancer Institute-funded research centers at universities, which emphasize cross-disciplinary projects, also “break down barriers between academia and industry, and enable us to bring cures and therapies to the market faster,” says Clark.

In fact, “to a certain extent, the lines between academia and industry are blurring,” says Strum. Hybrid institutes are being launched or expanded to harness the collaborative power that flourishes when industrial and academic scientists work together. COH is similar to an academic setting, yet students and postdocs are able to interact with clinicians, physicians, and sometimes patients, explains Malkas. Ph.D. committees are much more diverse compared with strictly academic institutions. For example, a standard cancer research dissertation committee at COH could include experts in bioinformatics, gene expression, and proteomics, whereas at a university, “the committees are more homogeneous,” she says.

These hybrid institutions play another important role since “the huge, prohibitive costs associated with the clinical development of very early and risky research make it difficult for industry to pursue truly novel and unique molecular targets for cancer therapy, diagnosis, or intervention,” describes Malkas. Organizations like COH recognize the value in this type of science. COH has “set up the infrastructure and resources to absorb the risk of early development...of discoveries not only for our own investigators, but for other institutions as well,” she says.

Universities are also taking the lead in designing new centers that aggregate research goals and accelerate new discoveries to market. The Broad Institute, where Eliasof worked before coming to industry, and the New York Genome Center are two examples. But even within typical academic departments, scholars are becoming savvier **continued>**



Cures don't just happen.

They demand collaboration. Dedication. Talent. Teamwork.

Job Opportunities – Computational Biology

The St. Jude Children's Research Hospital – Washington University Pediatric Cancer Genome Project (PCGP) is the largest investment to date aimed at understanding the genetic origins of childhood cancers. PCGP is establishing an on-site, Clinical Laboratory Improvement Amendments certified laboratory at St. Jude. This lab will use next-generation sequencing (NGS) to evaluate samples from St. Jude patients and will explore the optimal way to use this data in patient care. To support the clinical genomics initiative, we are recruiting for multiple positions in the Computational Biology Department.

The Computational Biology Department has a well-established track record in developing and delivering state-of-the-art computational methods for analyzing NGS data and for producing high-impact publications in top-tier journals. We are looking for highly motivated and talented bioinformatics scientists and engineers to work on competitive projects and solve challenging problems in genomic and epigenetic profiling of pediatric cancer for both research and clinical applications.

To learn more about current job opportunities and to apply, visit www.stjude.org/jobs

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Ranked in the top 10 best places to work in academia by The Scientist yearly since 2005.
Named the nation's No. 1 pediatric cancer care hospital by Parents magazine, 2009.
Named the nation's best children's cancer hospital by U.S. News & World Report, 2010.
Named to FORTUNE magazine's 100 Best Companies to Work For yearly since 2011.
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Peggy and Charles — STEPHENSON CANCER CENTER — at The University of Oklahoma

FACULTY POSITIONS

The Peggy and Charles Stephenson Cancer Center (SCC) at the University of Oklahoma Health Sciences Center invites applications for faculty positions. Rank and tenure eligibility to be commensurate with qualifications and experience. Interested applicants should have a demonstrated record of sustained, peer-reviewed funding and publications, with preference given to active NCI funding.

The SCC is Oklahoma's only academic cancer center. It has a well-developed and supportive infrastructure and over 120 members from the University of Oklahoma (OUHSC, OU Norman, OU Tulsa), the Oklahoma Medical Research Foundation and Oklahoma State University. The SCC places a high priority on promoting translational research that moves research ideas into clinical applications. Position applicants should have demonstrated research aligned with one of the following four SCC research programs (and research foci):

- Basic Cancer Biology (epigenetics/chromatin regulation; tumor microenvironment; tumor-stromal cell interactions; angiogenesis; metastasis; tumor initiating cells/cancer stem cells)
- Experimental Therapeutics (molecular targeted therapies; gene and drug delivery; nanomedicine; high-throughput drug discovery; cancer imaging; Phase 0 & Phase I trials)
- Gynecologic Cancers (angiogenesis; drug resistance)
- Cancer Health Disparities (special populations, with emphasis in American Indian; health outcomes; tobacco research)

With the support of a recently completed \$67 million fundraising campaign and institutional development grants of over \$30 million (cancer research) and \$5 million (tobacco prevention and control research) from the Oklahoma Tobacco Settlement Endowment Trust, the SCC has launched a major initiative to recruit 20 cancer-focused researchers over the next four years. SCC Shared Resources include a Biospecimen Acquisition Core and Bank, Molecular Imaging Core, Cancer Functional Genomics Core, Cancer Tissue Pathology Core, Biostatistics Core, Special Populations Core, and Proposal Services Core. The SCC has a large clinical trials research program and a rapidly expanding Phase I Program.

Applicants must possess a PhD, MD, or MD/PhD in a relevant discipline and have a demonstrated potential for excellence in research. Selected candidates will have an appointment in an academic department as well as the SCC. For additional information, please visit www.stephensoncancercenter.org.

Applicants should provide electronic copies (only) of a cover letter stating area of expertise and qualifications, synopsis of professional goals, research interests, *curriculum vitae*, and e-mail addresses for three references to CancerResearch@ouhsc.edu. Confidential enquiries may be addressed to **Danny N. Dhanasekaran, PhD, Deputy Director for Basic Research**, at danny-dhanasekaran@ouhsc.edu.

The University of Oklahoma is an Equal Opportunity Employer.

Featured Participants

CellAct Pharma
www.cellact.eu

Cerulean Pharma
ceruleanrx.com

City of Hope
www.cityofhope.org

Cleveland State University
www.csuohio.edu

David Geffen School of Medicine at UCLA
healthsciences.ucla.edu/dgsom

G1 Therapeutics
www.g1therapeutics.com/home.html

Genomic Health
www.genomichhealth.com

Johns Hopkins University (JHU) School of Medicine
www.hopkinsmedicine.org/som

John Theurer Cancer Center at Hackensack UMC
www.jtcancercenter.org

The University of Texas MD Anderson Cancer Center
www.mdanderson.org

WellPoint
www.wellpoint.com



“Researchers in any environment will have to become more outcome based.”

—Andre Goy

questions about the role of genomics in patient care,” he explains. “We are definitely seeing a broad need in medicine to find ways of using genomic techniques to make a clinical difference.”

Andre Goy, chief of the Lymphoma Division and director of the Tissue and Tumor Bank at the John Theurer Cancer Center at Hackensack University Medical Center (UMC), notes that in both academic and industrial labs there is more of a spotlight on understanding cancer cell biology using novel therapies, or “precision medicine” utilizing biomarkers identified through genomics. “The next generation of physicians and scientists will be exposed early on to this progress as we evolve towards a redefinition of the classification of cancers—not only based on the organ of origin but on shared molecular characteristics, hopefully providing a rationale for treatment decisions in a growing number of cancer patients,” he suggests. Furthermore, with an expanding number of

scientists working on both sides of the fence, Goy is seeing a heightened interest in the business of medicine, with an increased number of scientists and physicians pursuing MBAs. “Many factors are reshaping the field of cancer care,” he clarifies. The research techniques, technology, economics, and molecular diagnostics are just some of those moving pieces. Generally “researchers in any environment will have to become more outcome based,” says Goy.

Advice for Scientists in the Changing Landscape

One piece of advice that these experts keep emphasizing is that there is no set course for a career in cancer research. Scientists can now choose industry, academia, a hybrid, or a combination of any of these. Just as the field of cancer research is becoming more diverse and interdisciplinary, so is the profession.

“It’s not like once you make a choice, you’re stuck with that choice forever,” says Eliasof. “You can move from academia to industry and vice versa,” although he admits it can be harder to move from a company back to a university because of the lack of demonstrable publications. But there is a way to make a company-to-university transition easier and more efficient: “Keep interacting with your colleagues in academia so they know about you,” advises **Nalán Utku**, the managing director and co-founder of CellAct, a small German cancer research company who has also spent considerable time in academia.

But the academic setting is experiencing a metamorphosis in more ways than one. For instance, “academia is starting to recruit from industry more,” says Eliasof. And “the choices of direction of cancer research in academia are not as broad as they used to be,” notes Clark. “Academic research is being driven more and more by where the money comes from.”

As more researchers realize that cross-disciplinary and cross-sector investigations are good for science and medicine, leaders predict there will be more collaboration between industry and academia. “We’re discovering that we need each other,” says Malkas. “Young people today...need to learn that the old cowboy way of doing science where you work by yourself is gone.” Indeed, the majority of the time “it’s about ‘group science’ for groundbreaking research,” echoes Clark.

To prepare for a career in cancer research, scientists need a multidimensional skill set to tackle questions from different fronts. “Think big and bold, but focus,” advises Goy. “The old rules of research still apply, but the way we are going to do things will with no doubt change dramatically in the coming years.” Academic scientists should get to know clinicians and their work with patients, and learn business skills. To further enhance one’s marketability, develop a good understanding of data analysis and computational biology—skills which are becoming more and more coveted as technology improves and the amount of data being analyzed increases exponentially, notes **Giulio Draetta**, director of the Institute for Applied Cancer Sciences (IACS) and professor in the Department of Genomic Medicine at the University of Texas MD Anderson Cancer Center.

Above all, attitude is key. “Whether you are in industry or academia, research is a discipline that’s bound to give you 99% failure,” says Draetta. “You have to believe in yourself.”

Alaina G. Levine is a science writer and science careers consultant/speaker based in Tucson, AZ
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Faculty Positions in the Genetics of Aging at The Jackson Laboratory

The Jackson Laboratory is an independent, nonprofit, biomedical research institution with more than 1,400 employees, and three campuses located in Bar Harbor, Maine, Farmington, Connecticut and Sacramento, California. The Laboratory's mission is to discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health. We are actively expanding our research program in the genetics of aging and recruiting faculty members with a research focus in this field, especially those centered on cellular mechanisms of aging.

We seek scientists with Ph.D., and/or M.D. degrees, who have completed postdoctoral training and have a record of research excellence. Candidates should have the ability to develop a competitive, independently funded research program that takes advantage of the mouse as a genetic model for understanding human biology, diseases of aging and longevity.



Leading the search for tomorrow's cures

Open positions are:

Program Director — In addition to his/her research program, this experienced leader in the field of aging will promote and coordinate the activities of The Jackson Laboratory's aging program, and spearhead fund-raising efforts in this research area. The Director will lead The Jackson Laboratory Nathan Shock Center of Excellence in the Basic Biology of Aging. The Center focuses Jackson Laboratory researchers' diverse expertise in biology and genomics on problems of aging, leading to enhanced resources for the aging research community and a better understanding of the molecular mechanisms at work in lifespan and healthspan.

Apply to position #3636.

Assistant, Associate or Full Professor — Aging Genetics Faculty members will run independent labs for relevant biomedical research on the biology of aging and age-associated diseases, will participate in The Jackson Laboratory's aging programs, and will mentor graduate students, postdoctoral associates and research staff, in a collaborative and supportive environment.

Apply to position #3635.

Applicants must apply online by submitting a curriculum vitae and a concise statement of research interests and plans as one document, to www.jax.org/careers, referring to position #3635 or #3636. In addition, please arrange to have three letters of reference sent to: facultyjobs@jax.org.

The Jackson Laboratory is an OEO/AA Employer.

600 Main Street, Bar Harbor, ME, 04609 | www.jax.org



Seattle Children's
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Faculty Position in Developmental Biology and Regenerative Medicine

The Center for Developmental Biology and Regenerative Medicine in the Seattle Children's Research Institute and Department of Pediatrics at the University of Washington School of Medicine seeks outstanding scientists (up to three positions) to be appointed full-time, without tenure, at the Assistant, Associate or Professor level. Applicants should possess Ph.D., M.D., or M.D., Ph.D. degrees. Areas of interest are broad; these include human genetics, model organism genetics, stem cells and regeneration, and systems level analyses of pathways involved in disease and development. The successful applicant will enjoy a competitive start-up package, and excellent space and state-of-the-art facilities in the Research Institute, which is near the University of Washington South Lake Union Campus, the Fred Hutchinson Cancer Research Center, and the Institute for Systems Biology. He or she will be appointed as faculty at the University of Washington, and will be part of a Center with diverse interests and expertise.

Please send a CV, a 2-3 page description of research interests and plans, and contact information for 3 letters of recommendation to:

Mark Majesky, Ph.D., and David Beier, M.D. Ph.D.,
search committee co-chairs

c/o Wendy Kramer, Business Manager

Center for Development Biology and Regenerative Medicine
Seattle Children's Research Institute
1900 Ninth Avenue, MS: C9S-5
Seattle, WA 98101-1309
wendy.kramer@seattlechildrens.org

University of Washington faculty engage in teaching, research and service. In order to be eligible for University sponsorship for an H-1B visa, graduates of foreign (non-U.S.) medical schools must show successful completion of all three steps of the U.S. Medical Licensing Exam (USMLE), or equivalent as determined by the Secretary of Health and Human Services. The University of Washington is building a culturally diverse faculty and strongly encourages applications from female and minority candidates. The University is an Equal Opportunity Affirmative Action Employer.



STANFORD
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Stanford University Medical Center

Melanoma Investigator

Assistant Professor, Associate Professor, or Professor

The Division of Oncology in the Department of Medicine at Stanford University is recruiting a melanoma physician investigator. The faculty position is at the Assistant, Associate or Full Professor level in either the University Tenure Line (UTL) or the Medical Center Line (MCL), and the successful candidate should be an accomplished laboratory or clinical investigator. Faculty rank and line will be determined by the qualifications and experience of the successful candidate.

The predominant criterion for appointment in the UTL is a major commitment to research and teaching. The major criteria for appointment for faculty in the MCL shall be excellence in the overall mix of clinical care, clinical teaching, scholarly activity that advances clinical medicine, and institutional service—appropriate to the programmatic need the individual is expected to fulfill.

The successful candidate will be expected to build a strong clinical/translational research program, teach fellows, and participate in the melanoma oncology clinics of the interdisciplinary Pigmented Lesion and Melanoma Program. Candidates should have an MD or MD/PhD and be board certified or board eligible in Medical Oncology and/or Hematology. The Division of Oncology benefits from an outstanding scientific and clinical environment at Stanford, including active collaborations with the basic science departments and the Stanford Institutes, including the Stem Cell Institute, the Immunity, Transplantation, and Infection Institute, and the Cancer Institute. For more information about existing programs, see <http://cancer.stanford.edu/skincancer/expertise.html>.

Candidates should submit a detailed letter of interest and curriculum vitae to: **Margaret Wootton, Melanoma Search Committee, Division of Oncology, Stanford University, 265 Campus Drive, Room G3141, Stanford, CA 94305-5463; email: margaret.wootton@stanford.edu**

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the university's research, teaching and clinical missions.

Multiple Tenured Faculty Positions at Nankai University Chemistry and Material Science Divisions



With well internationally established recognition, the Chemistry and Material programs at Nankai University are inviting the applications for full-time tenured faculty positions of academic ranks of Assistant/Associate/Full Professor, to be appointed in Chemistry and Material divisions of Nankai University. The positions are available immediately. Applications will be evaluated by the faculty search committee upon receipt until the positions are filled.

Nankai University

As one of the top elite universities and comprehensive one in China, Nankai University has wide and international recognition. It is located in Tianjin City, one of the largest cities in China and connected with Beijing by 30 min high speed train. The Chemistry division of Nankai has been ranked as one of the top ones in China.

Qualifications

Candidates in all Chemistry and Material majors are welcomed. Successful candidates are required to have Postdoc/ faculty experience and strong proven academic records.

Salary/Benefits

Successful candidates will be provided with a sizable start-up package including competitive research funding, salary, initial housing/rental funding, and lab space. Successful candidates are also encouraged/supported to apply for various national programs such as "Thousand Talent Plan" and "ChangJiang Professorship" programs.

To Apply

Candidates are encouraged to initially email their CV with the publication list AND a one-page statement to **Prof Chen** at email yschen99@nankai.edu.cn.

FOCUS ON CANCER RESEARCH



Memorial Sloan-Kettering
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Director, David M. Rubenstein Center for Pancreatic Cancer Research Memorial Sloan-Kettering Cancer Center, New York, NY

Memorial Sloan-Kettering Cancer Center (MSKCC) and the Sloan-Kettering Institute are seeking qualified applicants for the position of Director to lead the newly formed David M. Rubenstein Center for Pancreatic Cancer Research. MSKCC is an NCI-designated comprehensive cancer center that is renowned for excellence in cancer research and clinical care.

The Director will oversee the clinical, translational, and scientific investigation of pancreatic cancer across the institution. He/she will lead the multidisciplinary collaboration of scientists, medical oncologists, surgeons, pathologists, radiologists, clinical geneticists, epidemiologists, and gastroenterologists. The incumbent will strengthen and expand existing research activities, attract new investigators to pancreatic cancer research, promote educational programs, and foster training opportunities.

The successful candidate will have: (1) an exceptional record of accomplishment in the field of pancreatic cancer research, (2) established leadership and mentorship skills, (3) a record of scholarly achievement and publication, and (4) a commitment to translational and collaborative research. Please forward a curriculum vitae and statement of interest by **April 26, 2013** to:

Clara Irizarry, MPA

**Manager, Office of Academic Recruitment, MH
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY 10021
212-639-5819**

e-mail: irizarre@mskcc.org

MSKCC is an Equal Opportunity and Affirmative Action Employer committed to diversity and inclusion of all aspects of recruiting and employment. All qualified individuals are encouraged to apply.



**Ocean Research center of Zhoushan,
Zhejiang University**

浙江大学舟山海洋研究中心

Ocean Research Center of Zhoushan, Zhejiang University is a research institution in Zhoushan City, Zhejiang Province, China, which is set up by Zhoushan government and Zhejiang University in 2009. Multiple positions for senior talents are open in following areas:

- Marine sciences, including physical oceanography, marine chemistry, marine biology, marine geology etc.
- Marine engineering, including shipbuilding, mechatronics, environment, information, energy etc.
- Marine policy and culture.

Applicants should have strong research profile and potential capacity to conduct innovative research in these areas and have at least 3 years working experiences in first-class universities, research institutes or enterprises. The center provides state-of-the-art research facilities and strong supporting staffs. Competitive start-up support, salary and benefits will be offered according to individual qualification and experience.

Please submit your full CV, the cover letter and your future plan to **Ji Wenxiu** at ajiwenxiu@yahoo.com.cn or call **86-(0580)-2186309**. The positions will be open until they filled by appropriate candidates.

VISITING-PROFESSOR, POST-DOCTORAL LONG-TERM COLLABORATION PROGRAM

The Oswaldo Cruz Foundation (Fiocruz; www.fiocruz.br), an institution affiliated to the Brazilian Ministry of Health, is building the Center for Technological Development in Health (CDTS for its acronym from Portuguese). Following its inauguration in 2014, the buildings of the CDTS will host technological platforms, animal experimentation and flexible laboratories in 20,000 m² of state of the art facilities. The CDTS will provide the necessary infrastructure to fully implement the spirit of the Brazilian 2004 Law on Innovation, which encourages partnerships between public and private sectors. Fiocruz has mandated the CDTS to establish and work in collaboration with other centers of scientific excellence for the joint development of health products against diseases that are of epidemiological or economic importance to Brazil, especially neglected tropical diseases.

To mobilize and strengthen the human resources needed, Fiocruz and CAPES (an agency of the Ministry of Education) have dedicated the following fellowships for PhD's to collaborate with the CDTS. In the long term the program aims to establish sustained partnerships between Fiocruz and other leading institutions, both public and private.

1. Post-doctoral Program

Open to Brazilian candidates who have completed their PhD/DSc work and are willing to perform post-doctoral training at public or private institutions in Brazil or abroad with a commitment to join a CDTS project upon the completion of the program.

- **RD-BR:** up to 20 (twenty) *Post-doctoral Fellowships* to train young Brazilian scientists in public or private R&D centers of excellence in Brazil (maximum 48 months);

- **PD:** up to 10 (ten) *Post-doctoral fellowships* to train young Brazilian scientists at institutions abroad (maximum 12 months).

2. Visiting Professor Program

Open to scientists and technology management professionals at PhD/DSc or equivalent level, from institutions of public or private sectors, with proven experience and outstanding achievement. Citizens from countries that maintain a diplomatic relationship with Brazil are eligible. The program is available for stays lasting from one week to one year and can be renewed. The work will be conducted at Fiocruz. Stipends will be determined based on experience.

- **PD-ES:** up to 10 (ten) *Visiting Professor Fellowships* to support senior Brazilian scientists to conduct research abroad (maximum 18 months);

- **PVE:** up to 10 (ten) *Visiting Professor Fellowships* to support foreign professionals for collaboration on projects within the CDTS and to participate in Fiocruz graduate programs (maximum 48 months);

- **PVNS:** up to 10 (ten) *Visiting Senior Professor Fellowships* to support senior Brazilian scientists to collaborate on projects within the CDTS and to participate in Fiocruz graduate programs (maximum 48 months).

Skills areas:

1. Research and Development: Structure and function of macromolecules (genomics, proteomics, glycomics, computational biology, bioinformatics) and small molecules (lipidomics, metabolomics); Disease-associated biological networks; Expression systems and fermentation (bacteria, yeast, mammalian, plant); Development of animal models (transgenics and knock-out/in); Bioprospecting and drug discovery; Nanotechnology; Biostatistics; Prevention/control of infectious diseases;

2. Technology Development: Molecular biomarker discovery and production: synthesis of peptides & DNA; Aptamer design; Production and purification of monoclonal antibodies and recombinant proteins; Pre-clinical studies including toxicology; Clinical pharmacology; High content screening; Bioassays; Libraries of chemicals and biologicals; Drug design, *in silico* modeling; Chemistry/Biology, Manufacturing and Control (CMC/BMC); Standard Operating Procedures (SOPs); Animal experimentation; Biosafety facilities

3. Technology Management: Intellectual property rights and patent landscape; Regulatory affairs – liaison with Regulatory Authorities, development of Drug Master Files (DMF), compilation and submission of dossiers (IND or NDA); Technology transfer;

4. Business Development: Pipeline strategy and portfolio management; Business planning; Partnership strategy; Negotiation of public-private alliances and partnerships; Management of established alliances and partnerships; Fund raising; Health technology assessment and economic evaluation in health;

5. Capacity building: multidisciplinary cross-cutting capacity strengthening for R&D, technology development, technology management and business development (for individuals, institutions and networks), built on national and international partnerships with industries, PDPs, regulatory agencies and global health initiatives for disease control; including risk management, knowledge management, research ethics, clinical development, product delivery and implementation research.

Selection criteria:

Priority will be given to eligible candidates who can prove:

1. Strong support for their applications from the institution where the Post-doctoral stay will be performed or where the Visiting Professor is affiliated, including willingness to engage in long-term collaboration with Fiocruz after the training or visiting period;

2. Relevance of the training or R&D activities to Fiocruz objectives and goals;

3. Previous experience pertinent to the CDTS project and its long-term goals.

**Candidates will be selected by a six-member expert panel nominated by Fiocruz and CAPES.
The program will run until 2016.**

For further information or to express interest, contact: *Center for Technological Development in Health (CDTS) Oswaldo Cruz Foundation (Fiocruz) - Ministry of Health of Brazil - Campus de Manguinhos - Av. Brasil 4.365 - Rio de Janeiro, RJ - CEP 21.040-900 - Brazil - E-mail: cdts@fiocruz.br (with copy to anaelisa@cdts.fiocruz.br)*

www.fiocruz.br



**Health and Human Services
National Institutes of Health
EUNICE KENNEDY SHRIVER NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
Program in Genomics of Differentiation
Postdoctoral Positions Available**

Fully funded postdoctoral positions are available in the Program in Genomics of Differentiation (PGD), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Health and Human Services (HHS), Bethesda, MD. The PGD is a diverse and highly interactive program in basic cellular, molecular, and developmental biology research at the NIH just outside of Washington, DC. The 19 laboratories making up the PGD encompass a wide variety of research areas, with strong emphasis on developmental patterning and differentiation, chromatin dynamics and epigenetics, the immune system, the viral life cycle, DNA replication, gene regulation, and RNA metabolism. Program investigators perform research using a wide variety of models including viruses, bacteria, mammalian cell culture, yeast, fruit flies, zebrafish, frogs, and mice. Environment, resources, and stipend support are excellent. For additional information on how to apply for a position in the PGD and to find out more information on its researchers see <http://science.nichd.nih.gov/confluence/display/pgd/Home>. Interested applicants with a M.D. or Ph.D., and less than 5 years' postdoctoral experience should send a CV, bibliography, cover letter with a brief description of research experience and interests, and the names of 3 references (with phone numbers) to individual investigators listed below.

Sohyun Ahn – Neurogenesis in the developing and mature nervous system of the mouse

Harold Burgess – Analysis of neural circuits controlling behavior in zebrafish

Mike Cashel – Molecular regulation of the prokaryotic stress response

Ajay Chitnis – Early neural and lateral line development, Notch signaling

David Clark – The role of chromatin structure in gene activation in yeast

Robert Crouch – RNase H function in health and disease

Igor Dawid – Early embryonic development and patterning in the frog and zebrafish

Melvin DePamphilis – Regulation of DNA replication in normal and in cancer cells in the mammal

Bruce Howard – Experimental and bioinformatic analysis of epigenome structure

Judith Kassiss – Gene silencing by *Polycomb* group genes (PcG) in *Drosophila*

James Kennison – *Drosophila* developmental genetics

Judith Levin – HIV-1 replication; reverse transcription, host restriction, and viral assembly

Paul Love – Genes regulating mammalian hematopoiesis

Todd Macfarlan – Epigenome reprogramming in mammals

Richard Maraia – RNA metabolism, the La protein and RNA Polymerase III

Keiko Ozato – Gene regulation in innate immunity

Karl Pfeifer – Epigenetic regulation of gene expression and cardiac development in mice

Thomas Sargent – Epidermal and neural crest development in frog and fish

Brant Weinstein – Vascular development in the zebrafish

The HHS and NIH are Equal Opportunity Employers.



**National Institute of
Arthritis and Musculoskeletal
and Skin Diseases**

Tenure-Track Investigator(s)

The Intramural Research Program (IRP) of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the National Institutes of Health (NIH) in the Department of Health and Human Services (DHHS) is recruiting outstanding tenure-track scientists (M.D., Ph.D. or M.D./Ph.D) in research areas relevant to musculoskeletal biology or diseases of Bone, Cartilage, or Muscle at the basic, translational or clinical level. Emphasis will be placed on the applicants' demonstrated track record of high-quality research and the originality and promise of their future plans. Successful applicants will be expected to develop energetic, creative, independent research programs within the highly interactive scientific environment in NIAMS, the NIH IRP, and the NIH Clinical Research center, located in Bethesda, Maryland.

Applicants should submit a cover letter that includes a short research interest statement (two page maximum), a CV with complete bibliography, and contact information for three referees. Applications will be considered beginning **May 1, 2013** until the position is filled.

Applications should be submitted to:

**Ms. Margaret Vincent – RE: Musculoskeletal Initiative
Building 31 Room 4C-12
9000 Rockville Pike, Bethesda MD 20892
Email: vincentm@mail.nih.gov**

DHHS and NIH are Equal Opportunity Employers.

The NIH is dedicated to building a diverse community in its training and employment programs.



**Medical College
of Georgia**

**Assistant, Associate, or Full Professor
of Neurobiology**

**Institute of Molecular Medicine and Genetics
Georgia Regents University**

Georgia Regents University (GRU) is accepting applications for an Assistant, Associate, or Full Professor position (tenure-track or tenured) in the Institute of Molecular Medicine and Genetics. Candidates should have a PhD or MD; postdoctoral experience; interests in neural development, synaptic plasticity, or disorders of the central or peripheral nervous systems; and a strong record of research accomplishments. Faculty members are expected to establish or have cutting edge research programs and participate in teaching medical and graduate students. GRU is a state supported academic medical center located in a historic city with outstanding recreational and lifestyle opportunities.

Please apply for this position at www.georgiahealth.edu/facultyjobs and reference position #9347. Submit a CV, statement of current/future research interests, and contact information for three references to: **Dr. Darrell Brann, c/o Deenie Cerasuolo (dcerasuolo@gru.edu)**. Applications will be received until the position is filled.

GRU is an EEO/AA/Equal Access Employer.

Postdoctoral Scholars

The University of Calgary is a vibrant community of scholars committed to discovering new knowledge and translating discoveries into applications that will benefit our society and the global community. Within this exciting research enterprise, the Faculty of Medicine is well positioned to improve health outcomes through innovation in research and education as well as meeting the health challenges of a growing society. With the launch of the University's Eyes High strategic vision, we are set to make ground-breaking contributions and invest in our future as leaders with a social responsibility to bring benefit to our society through excellence, integrity, and innovation. The University's Rising Star program recognizes the importance of postdoctoral fellows whose skills and perspective will define our success and shape our collective vision. For more information please visit <http://www.ucalgary.ca/risingstars/postdoc>

The Faculty of Medicine invites applications for new postdoctoral scholars in the strategic priorities of Brain and Mental Health, Infections, Inflammation and Chronic Disease, Engineering Solutions for Health and Human Dynamics in the Changing World with the following researchers:

Electron tomography and super resolution light microscopy, to examine the basis of transcription at the molecular level in real time.

Supervisor: Matthias Amrein, James McGhee, Karl Riabowol

Health services research within the area of integrative oncology: integrating and evaluating complementary therapies and education for cancer patients and health care providers.

Supervisor: Linda Carlson

Autologous stem cell therapy for skin regeneration and improved functional outcome following split thickness skin grafting.

Supervisor: Vincent Gabriel (Jeff Biernaskie)

Imaging the immune system to understand the molecular mechanisms underlying host response. Supervisor: Paul Kubes

Mechanisms of genomic instability in yeast and mammalian systems. Supervisor: Susan Lees-Miller

Structure and function of the NOD-like receptor (NLRP) subfamily of innate immune receptors in the pathogenesis of non-microbial/sterile inflammation. Supervisor: Justin MacDonald

How is household food insecurity defined and socially constructed as a policy problem in Canada. Supervisor: Lynn McIntyre

Assess mechanisms of inflammation in the gut and mechanisms of disease recovery and tissue resolution after injury.

Supervisor: Derek McKay

Glial and Schwann cell therapy based approaches to improve axonal regeneration and remyelination for nerve and spinal cord repair.

Supervisor: Raj Midha

Effects of Regular Exercise on Cerebrovascular Reserve in Older Adults: Role in the Prevention of Age-Related Cognitive Decline.

Supervisor: Marc Poulin

Regulation of Cerebral Blood Flow in Obstructive Sleep Apnea.

Supervisor: Marc Poulin

Nanomedicines for the treatment of autoimmunity.

Supervisor: Pere Santamaria

Biochemistry of EMMPRIN and extracellular matrix molecules.

Supervisor: V. Wee Yong

To advance the neuroscience of axon regeneration.

Supervisor: Doug Zochodne

Impact of perinatal environmental exposures, including exposure to neurotoxins and psychological stress, on children's neurodevelopment and behaviour. Supervisor: Deborah Dewey

Developing non-invasive assessment methods for bone strength and fracture healing using state-of-the-art high resolution computed tomography imaging. Supervisor: Steve Boyd

Applicants should clearly specify the supervisor/project that they are applying to including their curriculum vitae, a letter outlining their research experience, their vision for the research, and the contact information for at least three referees to the Faculty of Medicine, University of Calgary at medgrant@ucalgary.ca by April 20, 2013. Consideration of applications will continue until the positions have been filled.



西北农林科技大学

Northwest A&F University

Academic Talents Recruitment

Northwest A&F University, Shaanxi, China

Northwest A&F University (NWAUFU) is one of the key universities in China supported by the Central Government under the "Project 985" and "Project 211". It is located in Yangling, Shaanxi Province, the birth place of Chinese agricultural civilization. Founded in 1934 as the first higher education institution in modern agriculture and forestry, NWAUFU has achieved significant progresses and developments in the past 79 years. Now with its 23 colleges and departments, NWAUFU is the most comprehensive university in agriculture, forestry and hydro-science in China and one of the nation's premier research-oriented universities.

NWAUFU has established Special Professorships in the following academic areas and invites highly talented and motivated candidates to apply:

I. Academic areas with job openings

Maize genomics and molecular breeding,
Vegetable molecular breeding,
Tree genetics and breeding,
Animal molecular breeding,
Apple genomics,
Functional genomics of plant nutrition,
Crop functional genomics,
Soil chemistry,
Soil erosion prediction modeling,
Animal cell engineering and embryo engineering,
Pathogenic microorganisms of animals,
Plant stress biology,
Material cycling in watershed ecosystem,
Structural optimization and structure-function relationship of biologically active natural substances,
Food safety,
Theory and practical techniques for efficient crop irrigation,
Hydrological modeling theory and methods,
Agricultural machinery and technology,
Forestry economic theory and policy,
Contemporary sociological theory and research methods.

II. Applicant Eligibility

2.1 Ph.D. degree in related disciplines. Under 45 years old for applicants in natural sciences and under 50 years old for those in social sciences.
2.2 Holding an assistant professor or more senior position for international applicants. A rank of full professor or equivalent for domestic applicants.

III. Benefits and Salary

3.1 Entitlement of full professorship and supervision of Ph.D. students.
3.2 A minimal startup funding of ¥3 million for candidates in natural sciences and ¥1 million for those in social science.
3.3 An apartment and a ¥0.3 million household allowance will be provided. Ownership of the apartment will be transferred to the faculty members after serving the university for ten years.
3.4 In addition to regular salary and benefits, qualified applicants will be compensated with an annual allowance of ¥0.1-0.5 million. International candidates will receive a minimal annual salary of ¥0.6 million.
3.5 Spousal hiring will be accommodated.

IV. Application Procedure

4.1 Applicants should provide:
An English version of curriculum vitae with lists of publications, research interests and professional titles and activities;
A statement of research accomplishments and future teaching and research plan;
PDF copies of academic transcripts, diploma and degree certificate, five representative papers or books and documents for funded projects, awards, patents and keynote speakers at international conferences, etc;
Three recommendation letters with referrers' e-mails and telephone numbers.

4.2 NWAUFU will conduct a preliminary screen and invite eligible candidates to Yangling for an interview and campus visit. The selected candidates need to sign a contract with the university. **See our Chinese ad at <http://rcb.nwsuaf.edu.cn>**

In addition, NWAUFU the Global Experts Program and the Youth Global Experts Program are also open to applications from China and abroad. **Please visit our website: <http://rcb.nwsuaf.edu.cn> for more information.**

V. Contact

Address: No. 3 Taicheng Road, Yangling, Shaanxi Province, 712100 China.
Contact Person: Chen Xiaoyan
Tel: +86-29-87082855 87082577, Fax: +86-29-87082855
E-mail: rencaike@nwsuaf.edu.cn; rencaiban@gmail.com.
The university's website: <http://www.nwsuaf.edu.cn>



Charitable Foundation

The EGL Charitable Foundation invites you to apply to the Gruss Lipper Post-Doctoral Fellowship Program

Eligibility

- Israeli citizenship
- Candidates must have completed PhD and/or MD/PhD degrees in the Biomedical Sciences at an accredited Israeli University/Medical School or be in their final year of study
- Candidates must have been awarded a postdoctoral position in the U.S. host research institution.

Details regarding the fellowship are available
at www.eglcdf.org

NW210508R

PRIZES



The 2013 (29th) International Prize for Biology



Calling for Nominations

This year's research field: **Biology of Evolution**

Please access to: <http://www.jsps.go.jp/english/e-biol>

Deadline: **May 17, 2013**

- The International Prize for Biology was established in 1985 to commemorate the sixty-year reign of Emperor Showa and his longtime devotion to biological research.
- The Prize is awarded each year to an individual who has made an outstanding contribution to the advancement of basic research in a field of biology.
- The Prize shall consist of a medal and a prize of ten million yen.

Recent Years Prize Winners



2012
Dr. Joseph Altman
(Neurobiology)



2011
Dr. Eric H. Davidson
(Developmental Biology)



2010
Dr. Nancy A. Moran
(Biology of Symbiosis)



78th Cold Spring Harbor Symposium on Quantitative Biology

Immunity & Tolerance

Organized by:

Michel Nussenzweig, HHMI / The Rockefeller University
Anne O'Garra, MRC National Institute for Medical Research, UK
Stephen Smale, University of California, Los Angeles School of Medicine
David Stewart & Bruce Stillman, Cold Spring Harbor Laboratory

May 29 - June 3, 2013

Poster abstracts due: April 15

Topics

Stem cells and cell fate decisions
Regulation of immune cell development
Antigen receptor gene assembly and modification
Signal transduction
Regulation of lymphocyte function
Innate immune response and inflammation
Adaptive immunity
Mucosal immunity

Organ specific immunity
Immune regulation and tolerance
Autoimmunity and allergy
Immunity and cancer
Pathogen-immune system interactions
Vaccine development
Novel strategies to engineer/harness immunity

Rafi Ahmed, Emory University School of Medicine
Shizuo Akira, Osaka University, Japan
James Allison, HHMI/ Memorial Sloan Kettering Cancer Center
Frederick Alt, HHMI/Children's Hospital
David Baltimore, California Institute of Technology
Yasmine Belkaid, National Institutes of Health
Albert Bendelac, University of Chicago
Zami Ben-Sasson, Hebrew University Medical School, Jerusalem, Israel
Christophe Benoist, Harvard Medical School
Pamela Bjorkman, HHMI/California Institute of Technology
Meinrad Busslinger, Research Institute of Molecular Pathology, Austria
Doreen Cantrell, College of Life Sciences Dundee, UK
Rafael Casellas, National Institutes of Health
Jean-Laurent Casanova, The Rockefeller University
Ajay Chawla, University of California, San Francisco
Max Cooper, Emory University
Peter Cresswell, HHMI/Yale Medical School
Shane Crotty, La Jolla Institute for Allergy and Immunology
Mark Davis, HHMI/Stanford University School of Medicine
Richard Flavell, Yale University School of Medicine
Frederic Geissman, Centre for Molecular & Cellular Biology of Inflammation
Sankar Ghosh, Columbia University College of Physicians & Surgeons
Rudolf Grosschedl, Max Planck Institute of Immunobiology, Germany
William Jacobs, HHMI/Albert Einstein College of Medicine
John Kappler, HHMI/National Jewish Health
Lewis Lanier, University of California, San Francisco
Dan Littman, HHMI/NYU School of Medicine
Richard Locksley, University California San Francisco
Tak Mak, Ontario Cancer Institute, Canada
Philippa Marrack, National Jewish Center Denver
Diane Mathis, Harvard Medical School
Ruslan Medzhitov, HHMI/Yale University
Miriam Merad, The Mount Sinai Hospital School of Medicine
Kenneth Murphy, HHMI/Washington University
Cornelis Murre, University of California, San Diego
Gioacchino Natoli, European Institute of Oncology, Italy
Michel Nussenzweig, HHMI/Rockefeller University
Anne O'Garra, MRC National Institute for Medical Research, UK
Eric Pamer, Memorial Sloan-Kettering Cancer Center
M. Virginia Pascual, Baylor Institute for Immunology Research
William Paul, National Institutes of Health/NIID

Susan Pierce, National Institutes of Health
Hidde Ploegh, Whitehead Institute for Biomedical Research
Jonathan Powell, Johns Hopkins University School of Medicine
Fiona Powrie, University of Oxford, UK
Cristina Rada, MRC Laboratory of Molecular Biology, UK
Klaus Rajewsky, Harvard Medical School
Anjana Rao, La Jolla Institute for Allergy & Immunology
Lalita Ramakrishnan, University of Washington
David Raulet, University of California, Berkeley
Jeffrey Ravetch, The Rockefeller University
Ellen Rothenberg, California Institute of Technology
Alexander Rudensky, Memorial Sloan Kettering Cancer Center
Federica Sallusto, Institute for Research in Biomedicine, Switzerland
David Schatz, HHMI/Yale Medical School
Robert Schreiber, Washington University School of Medicine
Harinder Singh, Genentech
Stephen Smale, UCLA School of Medicine
Tadatsugu Taniguchi, University of Tokyo, Japan
Alexander Tarakhovsky, The Rockefeller University
Craig Thompson, Memorial Sloan Kettering Cancer Center
Carola Vinuesa, Australian National University, Australia
Ulrich von Andrian, Harvard Medical School
Harald von Boehmer, Dana-Farber Cancer Institute
Bruce Walker, Ragon Institute of MGH, MIT and Harvard
Hedda Wardemann, Max Planck Institute for Infection Biology, Germany
Art Weiss, HHMI/ University of California, San Francisco
Irving Weissman, Stanford University
Wayne Yokoyama, HHMI/Washington University

Registration, abstract submission and further information:

<http://www.cshl.edu/meetings> ♦ email: meetings@cshl.edu
phone: 516-367-8346 ♦ fax: 516-367-8845

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Poster credit: Catherine Dougherty

CONFERENCE



In partnership:



The European Cancer Congress 2013: The largest platform for practice-changing data in Europe

The recognised multidisciplinary setting of the Congress is once again providing ideal surroundings for participants to leverage knowledge, promote education and build awareness about oncology - placing the patient at the heart of all discussions.

The renowned quality of the Scientific Programme will guarantee a comprehensive, stimulating, rigorous and highly educational scientific experience, irrespective of your role or focus in oncology.

Abstract Submission Closing Date: 17 April 2013
eccamsterdam2013.ecco-org.eu

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WORKSHOPS

Latin American eScience Workshop 2013

Sponsored jointly by Microsoft Research and FAPESP
May 13 – 15, 2013 – São Paulo, Brazil

In Brazil, researchers have been working together to increase our understanding of tropical ecosystems, human impact on the environment, biogenetics, and biodiversity. These efforts are providing new opportunities to improve our capabilities in data-intensive research and strengthen the eScience research community. From May 13 to 15, 2013, we will host a special eScience Workshop in the city of São Paulo, Brazil. The event will bring together more than 150 participants, including students and researchers from all over the world, to explore collaboration and research opportunities in areas such as environmental sciences, bioenergy, biodiversity, health and digital humanities. More information about the workshop and the registration can be found at the workshop website.

<http://www.fapesp.br/eventos/latam2013>



Symposium wine & cheese

CSH Cold Spring Harbor Laboratory 2013 Meetings & Courses

www.cshl.edu/meetings

Summer/Fall Meetings

Meeting dates

Wiring the Brain

July 18 - 22

Metabolic Signaling & Disease: From Cell to Organism

August 13 - 17

Eukaryotic mRNA Processing

August 20 - 24

Mechanisms of Eukaryotic Transcription

August 27 - 31

Behavior & Neurogenetics of Nonhuman Primates

September 6 - 9

Eukaryotic DNA Replication & Genome Maintenance

September 9 - 13

Microbial Pathogenesis & Host Response

September 17 - 21

Fall Courses

Course dates

Programming for Biology

October 14 - 29

X-Ray Methods in Structural Biology

October 14 - 29

Computational & Comparative Genomics

November 6 - 12

Stem Cell Biology

September 24 - 28

Neurobiology of *Drosophila*

October 1 - 5

Cell Death

October 8 - 12

Genome Informatics

October 30 - November 2

Cell Biology of Yeasts

November 5 - 9

Precision Medicine: Personal Genomes & Pharmacogenomics

November 13 - 16

Harnessing Immunity to Prevent & Treat Disease

November 20 - 23

Plant Genomes & Biotechnology: From Genes to Networks

December 4 - 7

Rat Genomics & Models

December 11 - 14

Antibody Engineering & Phage Display

November 6 - 19

Advanced Sequencing Technologies & Applications

November 12 - 24

The Genome Access Course

April 21 - 23, July 18 - 20, November 17 - 19



Symposium picnic

CSH Asia 2013 Meetings

www.csh-asia.org



Tongli, CSH-Asia Dushu Lake Conference Center & Suzhou

Meetings

Meetings dates

Metabolism, Obesity & Obesity-associated Diseases

May 20 - 24

Plant Cell & Developmental Biology

June 17 - 21

Yersinia 11

June 24 - 28

Summer School: Computational & Cognitive Neuroscience

July 6 - 24

New Advances in Optical Imaging of Live Cells & Organisms

August 20 - 23

Cell Signaling in Metabolism, Inflammation & Cancer

September 2 - 6

Molecular Basis of Aging & Disease

September 9 - 13

Frontiers in Bioinformatics & Computational Biology

September 23 - 27

Genetic, Genomic, & Translational Studies of Human Leukemia

October 7 - 11

CSHA/ISSCR Joint Meeting on Stem Cells in Science & Medicine

October 14 - 17

Development, Function & Disease of Neural Circuits

October 21 - 25

Tumor Immunology & Immunotherapy

October 28 - November 1

Nuclear Receptors & Metabolism

November 4 - 8

Bacterial Infection & Host Defense

November 18 - 22



Beer & tea, CSH-Asia



Faculty Position Department of Cellular and Structural Biology School of Medicine

Applications are invited for a FACULTY POSITION in the Department of Cellular and Structural Biology, School of Medicine, at the University of Texas Health Science Center at San Antonio.

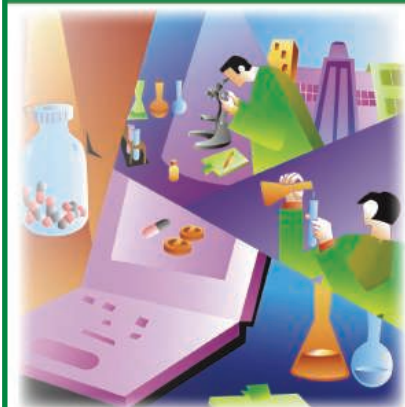
We seek applicants interested and experienced in teaching human histology courses, potentially coupled with an active research program in the area of cell biology. The ideal applicant will have training and experience in teaching human histology such that he/she could rapidly move into a course leadership role. Success in research, evidenced by publications and extramural support, would be an asset. Applicants who would complement current research strengths in the department, which include cancer biology, cell and organelle biology, DNA repair and mutagenesis, genetics and genomics, inflammation and disease, molecular biology of aging and age-related diseases, neurobiology and stem cell and developmental biology, are particularly encouraged to apply. Rank and tenure status will be dependent upon qualifications. Candidates should have a PhD, MD, DDS, or DVM. The Department has a rich history of teaching and research excellence and innovation. Priority will be given to applicants that show promise of enhancing these traditions. Potential applicants are invited to visit our web site <http://www.uthscsa.edu/csb/>.

The UT Health Science Center at San Antonio is home to the Nathan Shock Center for Excellence in Biology of Aging, an NCI-designated Cancer Center, an NIH-funded Clinical Translational Science Award and the Greehey Children's Cancer Research Institute. The UT Health Science Center is designated an Hispanic-Serving Institution. This is a wonderful opportunity for a new faculty member with an institutional administration focused on research, education and clinical care. The UT Health Science Center at San Antonio is a Tier One research institution located in the Northwest region of San Antonio and sits as a gateway to the picturesque Texas Hill Country. San Antonio is a dynamic and multicultural city with much to offer including an attractive cost-of-living.

Interested candidates should submit an application electronically as a single PDF comprised of their complete curriculum vitae, a statement of teaching philosophy and research interests, and a list of 4 to 5 references with contact information. Up to 3 high impact publications can be included in the application materials. Applications can be submitted to (email: Facsearchcsb@uthscsa.edu) and will be reviewed starting immediately, until the position is filled.

The University of Texas Health Science Center at San Antonio is An Equal Employment Opportunity/Affirmative Action Employer and is committed to diversity among its faculty, staff and students. All faculty appointments are designated as security sensitive positions.

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VCU

The Department of Anatomy and Neurobiology, School of Medicine, Virginia Commonwealth University is offering two tenure-track/tenured positions (F19890/F56750) at the Associate or full Professor level. The Department currently has 18 full-time neuroscience faculty. Internationally recognized research programs in **glial cell biology, neuroplasticity, and traumatic brain injury** are supported by substantial extramural funding. Additionally, the Department/VCU offers:

- An advanced, NIH-supported, bioimaging core housed within the department
- Multiple, staffed core facilities/centers (Center for Molecular Imaging, Flow Cytometry Resource Core, Chemical and Proteomic Mass Spectrometry Facility, Transgenic/Knock-out Mouse Facility, Cytogenetics Diagnostic Facility, and others)
- Positions that are supported by state funds
- Attractive and competitive salary and start-up funds

There is a large, active, and highly collegial neuroscience community at VCU that offers outstanding opportunities for collaborations with faculty in both basic science and clinical departments. Applicants should have an active and productive research program that complements the research directions of the department, and which is supported by significant extramural funding. The successful candidate will contribute to graduate and/or professional student teaching in either gross anatomy, histology, or neuroscience. Applicants should possess a PhD, MD, or DDS degree, or equivalent, and be committed to working with and fostering the development of a diverse faculty, staff, and student population or a commitment to do so as a faculty member at VCU. Review of the applications will begin immediately and the positions will remain open until filled.

Interested candidates should send a curriculum vitae, a one page letter of intent outlining their research and scholarly accomplishments, and the name, address, telephone number, and email address of three references. Application materials must be electronically submitted to anatrecruit@vcu.edu.

Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, minorities, and persons with disabilities are encouraged to apply.

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